NÁDIA CALVO MARTINS OKUYAMA

ANÁLISE DO POLIMORFISMO rs1801157 DE *CXCL12* NA INFECÇÃO PELO PAPILOMAVÍRUS HUMANO (HPV) E NO DESENVOLVIMENTO DE LESÕES CERVICAIS

Londrina
2017

NÁDIA CALVO MARTINS OKUYAMA

ANÁLISE DO POLIMORFISMO rs1801157 DE *CXCL12* NA INFECÇÃO PELO PAPILOMAVÍRUS HUMANO (HPV) E NO DESENVOLVIMENTO DE LESÕES CERVICAIS

Dissertação apresentada ao Programa de Pós-graduação em Patologia Experimental da Universidade Estadual de Londrina como pré-requisito para obtenção do título de mestre.

Orientadora: Profa. Dra. Karen Brajão de Oliveira

Londrina
2017

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

Okuyama, Nádia.
Análise do polimorfismo rs1801157 de CXCL12 na infecção pelo Papilomavírus humano (HPV) e no desenvolvimento de lesões cervicais / Nádia Okuyama. - Londrina, 2017.
73 f. : il.

Orientador: Karen Brajão de Oliveira.
Dissertação (Mestrado em Patologia Experimental) - Universidade Estadual de Londrina, Centro de Ciências Biológicas, Programa de Pós-Graduação em Patologia Experimental, 2017.
Inclui bibliografia.

1. Quimiocinas - Tese. 2. Infecção - Tese. 3. colo de útero - Tese. I. Brajão de Oliveira, Karen. II. Universidade Estadual de Londrina. Centro de Ciências Biológicas. Programa de Pós-Graduação em Patologia Experimental. III. Título.

NÁDIA CALVO MARTINS OKUYAMA

ANÁLISE DO POLIMORFISMO rs1801157 DE CXCL12 NA INFECÇÃO PELO PAPILOMAVÍRUS HUMANO (HPV) E NO DESENVOLVIMENTO DE LESÕES CERVICAIS

Dissertação apresentada ao Programa de Pós-graduação em Patologia Experimental da Universidade Estadual de Londrina como pré-requisito para obtenção do título de mestre.

BANCA EXAMINADORA

_____
Profa. Dra. Karen Brajão de Oliveira
Universidade Estadual de Londrina - UEL

_____
Profa. Dra. Carolina Batista Ariza
Universidade Estadual de Londrina - UEL

_____
Profa. Dra. Bruna Karina Banin Hirata
Universidade Estadual de Londrina - UEL

Londrina, 21 de março de 2017.

Dedico este trabalho às pessoas que mais me apoiaram durante meu mestrado e pelas quais tenho uma profunda admiração e respeito meus pais Getúlio Okuyama e Ivone Okuyama e meu companheiro William F. Recco.

AGRADECIMENTOS

À Profa. Dra. Karen Brajão de Oliveira, por ter me dado a oportunidade de estar em seu laboratório, sendo fundamental para o meu crescimento profissional e pessoal bem como pela orientação e dedicação neste trabalho.

Ao Programa de Pós-Graduação em Patologia Experimental, pelo incentivo à pesquisa e apoio institucional e à Capes, CNPq e Fundação Araucária, e a PROPPG-UEL, pelo apoio financeiro para realização deste estudo.

Aos Centros de Saúde Municipal Dr. Justiniano Clímaco da Silva (UBS Vivi Xavier) e Dr. Paulo Roberto Moita da Silva (UBS Armindo Guazzi), à Clínica de Colposcopia do CISMEPAR e ao Ambulatório de Ginecologia do HC/HU/UEL, pelo auxílio na obtenção das amostras. Um agradecimento especial às pacientes que aceitaram fazer parte deste projeto através da doação de suas amostras.

Aos membros da banca examinadora, Profa. Dra. Carolina Batista Ariza e Profa. Dra. Bruna Karina Banin Hirata, pela colaboração com a melhora deste trabalho.

Aos meus pais, Getúlio e Ivone, por acreditarem nas minhas decisões, por me acolherem nas minhas dificuldades, por não duvidarem da minha capacidade, pela compreensão, torcida e apoio nos momentos difíceis. Amo vocês!

Ao William, pelo incentivo diário, por me fazer sair da zona de conforto e encarar o desconhecido mundo do mestrado, por acreditar no meu potencial mais do que eu mesma, pelo carinho, amor e compreensão que foram fundamentais neste processo. Amo você!

Aos meus amigos de turma do mestrado, especialmente a Renata Streck, Giovana Carvalho e Alberto Sakaguchi, pelo conhecimento compartilhado dentro e fora da sala de aula, pelas risadas e pelos almoços e conversas no RU, por momentos que me propiciaram crescimento intelectual e pessoal.

Ao amigos de laboratório, especialmente a Nathália Tatakihara, Stephanie B. Garcia, Guilherme C. M. Cebinelli e Michelle M. Sena, por me ensinarem as metodologias utilizadas, por me ajudarem nas dificuldades, pela companhia nos dias de coleta e pelas longas conversas sobre a vida, pela parceria nos afazeres diários, pela companhia no almoço, por sempre me incentivarem e

acreditarem na minha capacidade. Ao Fernando C. dos Santos, Kleber Trugilo e Érica Romão pela parceria na escrita do artigo de revisão e montagem das figuras. À Fernanda B. Berti pela companhia durante as disciplinas e no laboratório. Ao Rodolfo Sanches pelo companheirismo na escala do laboratório. A todos vocês agradeço pelos momentos de descontração e por participarem tão ativamente do meu crescimento.

"Somos muito bons em preparar-nos para viver, mas não muito bons em viver a vida. Sabemos como sacrificar dez anos por um diploma e estamos dispostos a trabalhar arduamente para conseguir um trabalho, um carro, uma casa e assim sucessivamente. Mas temos dificuldade para lembrar que estamos vivos, no momento presente, o único momento para estarmos vivos " Thich Nhat Hanh

OKUYAMA, Nádia Calvo Martins. **Análise do polimorfismo rs1801157 de *CXCL12* na infecção pelo Papilomavírus Humano (HPV) e no desenvolvimento de lesões cervicais.** 2017**.** 72 f. Dissertação (Programa de Pós-Graduação em Patologia Experimental) – Universidade Estadual de Londrina, 2017.

RESUMO

O Papilomavírus Humano (HPV) é o principal agente etiológico no desenvolvimento de lesões cervicais que podem evoluir para o câncer cervical. Entretanto, somente o vírus HPV não é suficiente para o desenvolvimento das lesões e do carcinoma cervical. Neste contexto, mediadores imunes como as quimiocinas são importantes para o tráfego de leucócitos em processos biológicos como a inflamação e podem influenciar no desfecho da patologia. Dentre estas se destaca a quimiocina CXCL12, a qual apresenta um polimorfismo genético na região 3'UTR, denominado rs1801157 (g.17289G>A), cujo papel biológico no prognóstico do câncer é controverso. Até o momento não há estudos sobre a sua influência na infecção pelo HPV, assim como no desenvolvimento de lesões são escassos e contraditórios. Desta forma, o presente estudo teve por objetivo avaliar a associação deste polimorfismo com a infecção pelo HPV e no desenvolvimento de lesões cervicais. Dentre as 364 mulheres avaliadas, atendidas pelo Sistema Único de Saúde, o DNA viral foi detectado, em células do colo uterino por Reação em Cadeia da Polimerase (PCR), em 169 pacientes. O polimorfismo de *CXCL12* foi analisado através de PCR seguida de restrição enzimática (PCR-RFLP). A maior frequência do vírus foi observada em mulheres com idade inferior a 24 anos ($p<0,001$), solteiras ($p=0,002$), tabagistas ($p<0,001$) e com renda inferior a 1 salário mínimo ($p=0,040$), que apresentaram mais de 4 parceiros sexuais durante a vida ($p=0,007$) e mais de 5 gestações ($p=0,017$). Quando avaliado o papel do polimorfismo na infecção pelo HPV observou-se que o vírus foi mais prevalente em mulheres portadoras do alelo A ($p<0,001$), dado confirmado por meio da análise de regressão logística, ajustada para fatores de confusão: conhecimento sobre o vírus, renda mensal, tabagismo, estado civil, número de gestações, partos e de parceiros sexuais. Foi encontrada associação entre o polimorfismo e o desenvolvimento de lesão intraepitelial de alto grau (LIEAG) ($p=0,003$). Tendo em vista os dados apresentados, o polimorfismo de *CXCL12* rs1801157 está independentemente associado à infecção por HPV, podendo ser considerado como um marcador de susceptibilidade para a infecção. Contudo, mais estudos são necessários a fim de esclarecer o mecanismo pelo qual o polimorfismo contribui para a infecção e para a expressão da quimiocina no microambiente cervical, e qual o seu papel no desenvolvimento de lesões.

Palavras-chave: Quimiocinas. Infecção. Colo do útero.

OKUYAMA, Nádia Calvo Martins. **Evaluation of *CXCL12* rs1801157 polymorphism in Human Papillomavirus (HPV) infection and lesion development.** 2017. 72 p. Dissertação (Postgraduate Program in Experimental Pathology) – State University of Londrina, 2017.

ABSTRACT

Human papillomavirus (HPV) is the main etiologic agent in cervical lesions development that can lead to cervical cancer. However, only HPV virus is not sufficient for the development of cervix lesions and carcinoma. In this context, immune mediators such as chemokines are important for leukocyte trafficking in important biological processes as inflammation and may influence the pathology outcome. Among these chemokines, CXCL12 stands out, which presents a 3'UTR polymorphism, known as rs1801157 (g.17289G> A), whose biological role in cancer prognosis is controversial, and to date, there are no studies on its influence on HPV infection, as well as data and its role in lesion development are scarce and contradictory. In this context, the present study aimed to evaluate the association between this polymorphism on HPV virus infection and cervical lesions development. Among 364 women attended by the public health system, HPV-DNA was detected in cervical cells by Polymerase Chain Reaction (PCR) in 169 patients. *CXCL12* polymorphism was assessed through restriction fragment length polymorphism analysis (PCR-RFLP). The highest frequency of the virus was observed in women younger than 34 years ($p < 0.001$), single ($p = 0.002$), smokers ($p < 0.001$) and with a monthly income lower than 1 minimum wage ($p = 0.040$), more than 4 sexual partners during the lifetime ($p = 0.007$), and more than 5 pregnancies ($p = 0.017$). When evaluated the association of polymorphism in HPV infection, it was observed that HPV was more prevalent in women carrying the A allele ($p < 0.001$), as confirmed through logistic regression analysis, adjusted for confounder factor such as knowledge of the virus, monthly income, smoking, marital status, number of gestations and number of sexual partners. It was also observed an association between the polymorphism and high-grade squamous intraepithelial lesion (HSIL) development ($p=0.003$). According to our results, the *CXCL12* rs1801157 polymorphism is independently associated with HPV infection, being a promise candidate to HPV infection susceptibility biomarker. However, further studies are needed to clarify the mechanism by which the polymorphism contributes to infection and chemokine expression in the cervical microenvironment and its role in lesion development.

Keywords: Chemokine. Infection. Cervix.

LISTA DE SIGLAS E ABREVIATURAS

3'UTR 3'	*Untranslated Region*
ACKR3	*Atypical Chemokine Receptor 3*
AIDS	*Acquired immunodeficiency syndrome*
AIP4	*Atrophin interacting protein 4*
AKT	*Protein kinase B*
APC	*Antigen presenting cell*
ASCUS	*Atypical Squamous Cells of Undetermined Significance*
Bcl-2	*B cell lymphoma 2*
bFGF	*Basic fibroblast growth factor*
BMDC	*Bone marrow-derived cells*
CAF	*Lymphocytes, cancer-associated fibroblasts*
cAMP	*Cyclic adenosine monophosphate*
CCL2	*Chemokine ligand (family CC) 2 C*
CCL5	*Chemokine ligand (family CC) 5*
CCL7	*Chemokine ligand (family CC) 7*
CCL8	*Chemokine ligand (family CC) 8*
CCL20	*Chemokine ligand (family) 20*
CCR2	*Chemokine receptor (family CC) 2*
CCR6	*Chemokine receptor (family CC) 6*
CIN	*Cervical intraepithelial neoplasia*
CISMEPAR	Consórcio Intermunicipal de Saúde do Médio Paranapanema
CEP-UEL	Comitê de Ética em Pesquisa – Universidade Estadual de Londrina
CSCC	*Cervical squamous cell carcinoma*
CXCL5	*Chemokine ligand (family CXC) 5*
CXCL8	*Chemokine ligand (family CXC) 8*
CXCL11	*Chemokine ligand (family CXC) 11*
CXCL12	*Chemokine ligand (family CXC) 12*
CXCR4	*Chemokine receptor (family CXC) 4*
CXCR7	*Chemokine receptor (family CXC) 7*
DAG	*Diacylglycerol*
DNA	*Desoxyribonucleic acid*
dNTP	*Deoxynucleotide triphosphate*
E	*Early*
E2F	*E2 promoter-binding factor*
E6AP	*E6-associated protein*
EDTA	*Ethylenediaminetetraacetic acid*
EGFR	*Epidermal growth factor receptor*
ER	*Estrogen receptor*
ERK1/2	*Extracellular signal-regulated kinases ½*

GAG	*Glycosaminoglycan*
GATA 2	*GATA binding protein 2*
G-CSF	*Colony-stimulating factor*
GDP	*Guanosine diphosphate*
GEF	*Exchange factor of guanine nucleotide*
GIRK	*G-protein-coupled inwardly rectifying potassium*
GTP	*Guanosine triphosphate*
GPCR	*G protein coupled receptor*
HECT	*Homologous to E6AP carboxy terminus*
HIV	*Human Immunodeficiency Virus*
HSIL	*High-grade intraepithelial lesion*
HPV	*Human Papillomavirus*
HPV-AR	HPV de alto risco
HPV-BR	HPV de baixo risco
HPV-RI	HPV de risco indeterminado
HR	*High risk*
HSC	*Hematopoietic stem cells*
ICL	*Idiopathic CD4 lymphopenia*
IL17	*Interleukin-17*
INCA	Instituto Nacional do Câncer
IP3	*Inositol 3-trisphosphate*
I-TAC	*Interferon-inducible T-cell chemoattractant*
JAK	*Janus kinase*
JNK	*c-Jun N-terminal kinase*
L	*Late*
LCR	*Long control region*
LIEAG	Lesão intraepitelial de alto grau
LIEBG	Lesão Intraepitelial de baixo grau
LSIL	*Low-grade squamous intraepithelial lesion*
LTCD4	*Lymphocyte T CD4*
MAPK	*Mitogen activated protein kinase*
M-CSF	*Macrophage Colony-Stimulating Factor*
MMP2	*Matrix metalloproteinase 2*
mRNA	*Messenger RNA*
MMP2	*Matrix metalloproteinase 2*
MSC	*Mesenchymal stem cells*
NIC	*Neoplasia Intraepitelial Cervical*
NK	*Natural Killer*
NF-κB	*Nuclear Factor Kappa B*
NSCLC	*Non-small cell lung cancer*
ORF	*Open reading frames*
P16INK4a	*Cyclin-dependent kinase inhibitor*

p53	*Tumor suppressor protein 53*
PCR	*Polymerase chain reaction*
PCR-RFLP	*Restriction fragment length polymorphism*
PBSF	*Pre-B-cell growth stimulating factor*
PI3K	*Phosphoinositide 3-kinase*
PIP2	*Phosphatidylinositol 4,5-bisphosphate*
PKC	*Protein kinase C*
PLC	*Phospholipase C*
PMN	*Polymorphonuclear leukocytes*
pRB	*Retinoblastoma protein*
preDC-1	*Dendritic cell precursor-1*
preDC-2	*Dendritic cell precursor-2*
PTX	*Pertussis toxin*
SDF-1	*Stromal cell-derived factor 1*
SNP	*Single nucleotide polymorphism*
Sp1	*Specificity protein 1 transcription factor*
STAT	*Signal transducer and activator of transcription*
TAM	*Tumor-associated macrophages*
TAN	*Tumor-associated neutrophils*
TCR	*T cell receptor*
TE	*Tris-HCL-EDTA*
USP14	*Ubiquitin-specific protease 14*
VEGF	*Vascular endothelial growth factor*
WHIM	*Warts, hypogammaglobulinemia, infections, myelokathexis*

SUMÁRIO

1. INTRODUÇÃO

O Papilomavírus humano (HPV) foi descoberto por Harald zur Hausen nos anos 80, e desde então tem sido associado ao desenvolvimento de verrugas genitais (condilomas acuminados) ou ao desenvolvimento de lesões no colo do útero. No entanto, essas lesões podem regredir espontaneamente, devido ao *clearance* pelo sistema imunológico, ou então, se a infecção persistir, estas podem progredir dando origem ao carcinoma de colo uterino. Existem mais de 200 tipos de HPV que podem ser classificados, de acordo com seu potencial carcinogênico, em alto risco (HPV-AR), risco indeterminado (HPV-RI) e baixo risco (HPV-BR), estes últimos, responsáveis pelo condiloma acuminado (WOODMAN et al., 2007). A grande maioria dos casos de câncer de colo de útero está associada aos tipo virais de alto risco HPV-AR 16 e 18 (SMITH et al., 2007).

O câncer cervical é considerado um evidente problema de saúde pública mundial, sendo o terceiro tipo de câncer mais frequente entre as mulheres brasileiras e o quarto entre mulheres em todo o mundo (BRASIL, 2016; TORRE; BRAY; SIEGEL, 2015). Esta patologia está fortemente associada ao HPV, que está presente em 99% dos casos de cânceres (WALBOOMER, et al., 1999) O vírus HPV pode ser responsável também, por outros tipos de câncer como o de vulva (LEE et al., 2016), vagina (LEVOVITZ et al., 2014), ânus (MAI et al., 2015) e orofaringe (EGAWA et al., 2015), bem como por patologias benignas como verrugas genitais e papilomatose respiratória (ARBYN et al., 2012).

2. PAPILOMAVÍRUS HUMANO (HPV)

O papilomavírus humano é um vírus pequeno, não envelopado e seu material genético é composto por uma dupla fita de DNA (ácido desoxirribonucleico). Seu genoma contém 8000 pares de bases que se encontram organizados em 8 regiões de leitura abertas, também conhecidas como *open reading frame* (ORF) e uma região não-codificante conhecida como *long control region* (LCR). As ORFs contém 7 regiões chamadas de *early* (regiões de expressão precoce) que são responsáveis pela replicação viral e duas regiões denominadas *late* (regiões de

expressão tardia) que têm a função de codificar o capsídeo proteico (DOOBAR, 2007).

O vírus HPV pode permanecer na forma episssomal ou pode se integrar ao genoma da célula hospedeira, em fases mais avançadas da infecção. No estado episssomal, a proteína E2 regula a expressão das proteínas E6 e E7, suprimindo sua expressão. A integração do DNA viral ao genoma da célula hospedeira promove com a ruptura da região E2, o que interrompe a expressão da proteína E2, causando uma desregulação dos genes de expressão precoce, incluindo E6 e E7, bem como um aumento da capacidade proliferativa, uma etapa crucial na progressão para o câncer (MOODY; LAIMINS, 2010).

A participação da proteína E4 não está completamente elucidada e é expressa primariamente durante os estágios tardios da infecção ou no momento em que a amplificação do genoma é iniciada. A proteína E4 parece estar relacionada a liberação de novos vírus e a sua transmissão. O gene da proteína E5 parece ser conservado em muitos tipos de HPV. Este gene tem um papel importante no ciclo de vida do vírus e está envolvido com os oncogenes E6 e E7 (DOOBAR, 2013). As proteínas E6 e E7 estão fortemente expressas em tumores anogenitais malignos (DOOBAR, 2006; DIMAIO; PETTI, 2013). A proteína E7 liga-se ao retinoblastoma (RB), responsável pela regulação do ciclo celular. Uma vez hipofosforilada, a pRB libera o fator de ligação de E2 (E2F) no núcleo, permitindo a entrada da célula na fase S do ciclo celular. Enquanto que a proteína E6 interage com a ubiquitina-ligase E6AP e direciona sua atividade ao supressor tumoral p53, importante molécula de controle da apoptose, levando a sua degradação pelo proteassoma (BEAUDENON; HUIBREGTSE, 2008).

Além disso, E6AP pode atuar como coativador para o receptor de estrógeno (ER), o qual fosforilado liga-se a E6AP formando um complexo que é recrutado para ativar a transcrição de um subgrupo de genes promotores do receptor de estrógeno (ZHOU; SLINGERALND, 2014). O hormônio estrógeno contribui para o desenvolvimento do câncer de colo de útero através de seu receptor α. Este eixo hormônio-receptor é recrutado para a gênese do câncer cervical e sua persistência, uma vez que o estrógeno parece aumentar a expressão dos oncogenes E6 e E7 (SCHIFFMAN et al., 2013; ZUR HAUSEN, 2009). Neste sentido, a participação de E6AP como coativador do receptor de estrógeno e na proteólise de p53, sugere que esta sinalização via receptor de estrógeno poderia participar de uma conversa

cruzada com os mecanismos da carcinogênese desencadeada pelo HPV devido ao E6AP.

O HPV é transmitido pelo contato pele-pele, mucosa-mucosa e pele-mucosa. Diferentes tipos de HPV podem ser transmitidos ao mesmo tempo devido à via comum de transmissão (ZUR HAUSEN, 2009). Fatores de risco como idade da primeira relação sexual, número de parceiros sexuais, não uso de preservativo, uso de contraceptivo oral, múltiplos partos e tabagismo são importantes para o desenvolvimento de lesões relacionadas ao HPV, porém o motivo pelo qual eles funcionam como cofatores não está bem estabelecido (SCHIFFMAN et al., 2007). A infecção pelo HPV ocorre através de micro-lesões na camada basal do epitélio do colo do útero, no qual o vírus infecta a célula através de receptores ainda não estabelecidos. As células basais infectadas migram para as camadas superficiais do epitélio onde começam a se diferenciar. O DNA viral é então empacotado e novos vírus são liberados das células. A minoria das mulheres apresenta infecção persistente pelo HPV. Quando a infecção persiste, pode ocorrer o desenvolvimento de lesão intraepitelial de baixo grau (LIEBG) que por sua vez, pode evoluir para lesão intraepitelial de alto grau (LIEAG) podendo regredir ou então, posteriormente, progredir para o desenvolvimento câncer de colo de útero (BRUNI et al., 2010).

Diversos sistemas são adotados para classificar as lesões do colo do útero. O sistema que classifica o exame histopatológico (classificação de Richart, 1967) leva em consideração diferentes graus de neoplasia intraepitelial cervical (NIC), pode ser caracterizado por NIC 1, que apresenta displasia leve em apenas um terço do epitélio normal; NIC 2 apresenta displasia moderada até dois terços do epitélio normal e NIC 3 apresentando displasia severa e carcinoma *in situ*. Para classificar o exame citológico, utiliza-se o sistema Bethesda, no qual as lesões de colo uterino são classificadas em lesão intraepitelial de baixo grau (LIEBG) e alto grau (LIEAG) e ainda, adenocarcinoma *in situ*. LIEBG corresponde a NIC1, ao passo que LIEAG corresponde a NIC 2 e 3 (BRASIL, 2012).

Contudo, somente a infecção por vírus HPV-AR não é suficiente para promover a imortalização celular e sua malignidade. Modificações genéticas no hospedeiro devido à integração viral, mutações químicas e físicas podem contribuir para o processo (ZUR HAUSEN, 2009). Fatores endógenos e exógenos como o uso de tabaco, paridade, uso de contraceptivo oral (CASTELSAGUÉ; MUÑOZ, 2003), sistema imunológico deficiente e interações imunológicas no sítio da infecção

(PATEL; CHIPLUNKAR, 2009) podem influenciar a progressão da infecção pelo HPV para lesões relacionadas.

Neste contexto, podemos evidenciar as quimiocinas, uma vez que estudos sugerem que estas moléculas são reguladores importantes no desenvolvimento de infecções virais (MBEUNKUI; JOHANN, 2010) e também são responsáveis por controlar a migração celular, particularmente de leucócitos durante a inflamação, a qual se prolongada pode facilitar a carcinogênese por promover um microambiente ideal para o desenvolvimento e crescimento da célula tumoral (VANDERCAPELLEN et al., 2008).

3. QUIMIOCINAS E O MICROAMBIENTE TUMORAL

O termo quimiocinas foi elaborado em 1992 no Encontro Internacional de Imunologia em Budapeste (BAGGIOLINI, 2001). Quimiocinas são proteínas pequenas do sistema imunológico que possuem atividade quimiotática, estimulando a migração de diferentes tipos celulares como linfócitos, monócitos, neutrófilos, células endoteliais, células-tronco mesenquimais e células epiteliais malignas. Constituem uma grande família de citocinas com aproximadamente 50 quimiocinas endógenas em humanos e camundongos. As quimiocinas são divididas em 4 subfamílias baseados na posição dos primeiros resíduos de cisteína, denominadas: CC, CXC, CX3C e XC (BAGGIOLINI, 2001).

Os receptores de quimiocinas constituem uma grande subfamília tipo rodopsina, compostos por 7 domínios transmembranares. Os receptores são expressos em todos os leucócitos e podem ser divididos em dois grupos: receptores de quimiocinas acoplados a proteína G (GPCR) e receptores atípicos de quimiocinas, que parecem modular o gradiente de quimiocinas diminuindo a inflamação através do sequestro de quimiocina de maneira independente da proteína G. Existem aproximadamente 20 receptores de quimiocinas sinalizantes e 5 não-sinalizantes (BAGGIOLINI, 2001; GRIFFTHI et al., 2014).

Tecidos normais cuidadosamente controlam a produção e liberação de moléculas que induzem a progressão do crescimento e ciclo celular mantendo a homeostasia no número de células, a manutenção da arquitetura tecidual e sua função (POZZOBON et al., 2016). Diversos estudos têm demonstrado que as

quimiocinas e seus receptores estão envolvidos no crescimento e progressão tumoral como revisado por WANI et al., 2014.

Outro mecanismo mediado por quimiocinas que contribui para a tumorigênese é a angiogênese, necessária para permitir a propagação e progressão do tumor e indução da vascularização tumoral. Como os tecidos normais, o tecido tumoral também necessita de um suprimento de oxigênio adequado, metabólitos e uma forma eficiente de remover moléculas que não são necessárias. Dentre os diferentes tipos de câncer, estas necessidades podem variar e mudar de acordo com a progressão do tumor (BERGERS; BENJAMIM, 2003).

A progressão do tumor em direção a metástase é descrita como um processo de múltiplos estágios no qual células malignas saem do tumor original e vão colonizar órgãos distantes. A sequência básica da metástase pode ser definida em invasão local, sobrevivência na circulação, extravasamento e colonização, o que tem ajudado a racionalizar o complexo conjunto de propriedades biológicas que devem ser adquiridas para um tumor maligno se tornar uma doença metastática (NGUYEN et al., 2009). As células tumorais podem circular por todo o organismo e o fazem sob a influência de sinais que determinam o comportamento migratório. O processo metastático é dividido em dois estágios: primeiro, as células devem responder a sinais quimiotáticos que as encaminham a um local propício para a sua instalação; e segundo, elas devem sobreviver e prosperar na sua chegada. Evidências que apontam para o papel das quimiocinas na formação de metástases são provenientes da observação que as células tumorais expressam receptores de quimiocinas e que a expressão destes receptores não é aleatória (ZLOTNIK; YOSHIE, 2012).

As quimiocinas são mediadores-chave não somente no crescimento do tumor, formação de vasos sanguíneos e metástases, mas também no recrutamento de diferentes tipos celulares para o microambiente tumoral, incluindo macrófagos, neutrófilos, fibroblastos associados a tumores, linfócitos, células-tronco mesenquimais e células endoteliais (BALKWILL, 2004).

Dentre as quimiocinas, podemos destacar a CXCL12, que apresenta função quimioatraente para linfócitos, monócitos e células hematopoiéticas, sendo constitutivamente expressa por diversos órgãos como fígado, pulmão, linfonodos e cérebro (BLEUL et al., 1996). Sendo assim, tendo em vista a grande importância do papel biológico da quimiocina CXCL12, faz-se necessário um melhor estudo sobre

esta molécula e suas funções na infecção pelo HPV e na patogênese das lesões cervicais.

4. POLIMORFISMO rs1801157 DE *CXCL12*

O tipo mais comum de variação genética em seres humanos é denominado de polimorfismo de nucleotídeo único (do inglês, single nucleotide polimorphism (SNP)) e consiste na troca de um único nucleotídeo na sequência de DNA. Está presente em 1% ou mais da população e ocorrem, em média, a cada 300 nucleotídeos, sugerindo a existência de 10 milhões de SNP no genoma humano (VIGNAL et al., 2002). O risco para o desenvolvimento de doenças como câncer e a resposta individual a drogas já foram associadas a SNP. Quando o polimorfismo está presente em regiões codificantes do gene, este pode alterar a conformação e função proteica, enquanto SNPs presentes em regiões não codificantes do gene, podem alterar quantitativamente o produto da transcrição gênica (CHAKRAVATI, 2001; FAREED; AFZAL, 2013).

O gene da quimiocina *CXCL12*, está localizado a posição 10q11.1 e foi mapeado por hidridização *in situ* (SHIROZU et al., 1995). Este gene foi clonado pela primeira vez de uma linhagem celular derivada da medula óssea e posteriormente identificado como fator estimulante de crescimento de células pré-B. O gene *CXCL12* contem um SNP na região 3’ não traduzida (3’UTR) identificado como rs1801157, G801A ou SDF-1 3’A, no qual ocorre a troca de uma guanina por uma adenina, foi descrito pela primeira vez por Cheryl Winkler em um estudo com pacientes com AIDS (Síndrome da Imunodeficiência Adquirida) (WINKLER et al., 1998). O polimorfismo rs1801157 de *CXCL12* foi associado a um possível efeito protetor nos estágios tardios do HIV (vírus da imunodeficiência adquirida) na população brasileira (REICHE et al., 2006) e a um elevado risco no desenvolvimento de diversos tipos de câncer incluindo mama e linfoma (DE OLIVEIRA et al., 2009).

O papel biológico do polimorfismo rs1801157 de *CXCL12* no prognóstico de diferentes tipos de câncer é controverso. Uma frequência significativa do genótipo AA deste polimorfismo foi observada em pacientes com carcinoma de células renais e a taxa de sobrevida foi menor quando comparada a pacientes que apresentavam os genótipos GG e GA (CAI et al., 2013). De acordo com Schimanski

et al. (2011), pacientes portadores dos genótipos AA e GA são mais susceptíveis ao desenvolvimento de metástases distantes no câncer gastroesofágico. Enquanto que Razmkhah et al. (2005) demonstraram que este polimorfismo está associado a um aumento na susceptibilidade para o desenvolvimento de câncer de mama. Sei et al (2001) verificaram que *CXCL12* pode estar superregulado devido ao polimorfismo rs1801157, que pode influenciar nos níveis da quimiocina. Entretanto, De Oliveira et al. (2011) observaram que pacientes com câncer de mama portadoras do alelo A, apresentaram expressão de RNAm de *CXCL12* 2,1 vezes menor do que pacientes com o genótipo GG, sugerindo que o alelo A está associado a baixa expressão de *CXCL12* no sangue periférico em pacientes com câncer de mama receptor de estrógeno positivo. A combinação entre baixos níveis plasmáticos da quimiocina e o polimorfismo rs1801157 poderia identificar pacientes com um pior prognóstico no câncer de mama. Deste modo, os níveis de CXCL12 pode ser um importante biomarcador no prognóstico do câncer (HASSAN et al., 2008).

Não existem dados na literatura em relação a quimiocina CXCL12 e seu polimorfismo associado à infecção pelo HPV e ao desenvolvimento de lesões cervicais. Em relação ao câncer de colo de útero, os dados existentes são escassos e controversos. Segundo Tee et al. (2012), o polimorfismo rs1801157 não está associado ao risco de desenvolvimento de câncer de colo de útero. Da mesma maneira, Maley et al. (2010) investigaram mulheres com carcinoma escamoso invasivo e adenocarcinoma *in situ*. A conclusão do estudo foi que este polimorfismo não está relacionado ao desenvolvimento de câncer de colo de útero. Em contrapartida, o alelo A d doeste polimorfismo pode ser um fator de risco para o desenvolvimento de neoplasia de colo uterino em pacientes tabagistas (ROSZAK et al., 2015). Estes trabalhos não apresentaram dados relacionados à associação deste polimorfismo a infecção pelo HPV e ao desenvolvimento de LIEBG e LIEAG. Diante disto, este estudo objetivou investigar o papel deste SNP na infecção pelo HPV e no desenvolvimento de lesões cervicais que podem posteriormente progredir para o câncer de colo de útero.

5. OBJETIVOS

5.1 OBJETIVO GERAL

O objetivo geral deste estudo foi avaliar a influência do polimorfismo rs1801157 da quimiocina CXCL12 na infecção por HPV e no desenvolvimento de LIEBG e LIEAG, bem como verificar a presença do vírus em mulheres atendidas pelos programas de prevenção ao câncer de colo do útero do setor público de saúde da região Norte do Paraná.

5.2 OBJETIVOS ESPECÍFICOS

- Fazer um levantamento bibliográfico para produção de um artigo de revisão da literatura;

- Avaliar o perfil sociodemográfico das mulheres atendidas pelos programas de prevenção ao cânder de colo do útero do Sistema Único de Saúde (SUS) na região Norte do Paraná;

- Correlacionar a infecção pelo HPV e a presença das lesões provocadas por ele com dados sociodemográficos das mulheres atendidas, como faixa etária, estado civil, grau de escolaridade, renda familiar e tabagismo;

- Correlacionar a infecção por HPV e a presença das lesões provocadas por ele com as variáveis sexuais e reprodutivas, como idade da menarca, idade da primeira relação sexual, uso de métodos contraceptivos, número de parceiros sexuais ao longo da vida e nos últimos seis meses, número de partos e tipo(s) de parto(s) realizado(s).

- Avaliar a frequência dos alelos e genótipos do polimorfismo rs1801157 de *CXCL12* na população feminina atendida pelo programa de preveção ao câncer de colo de útero na região Norte do Paraná;

- Avaliar a associação do polimorfismo com a infecção pelo HPV e desenvolvimento de lesão no colo do útero da população feminina atendida pelo programa de prevenção ao cancer de colo de útero na região Norte do Paraná.

6. PRODUÇÃO CIENTÍFICA

6.1 ARTIGO 1

AIMS Medical Science, 3(4): 417–440.
DOI: 10.3934/medsci.2016.4.417
Received 17 October 2016,
Accepted 22 December 2016,
Published 26 December 2016

http://www.aimspress.com/journal/medicalScience

Review

Involvement of CXCL12 Pathway in HPV-related Diseases

Nádia C. M. Okuyama, Fernando Cezar dos Santos, Kleber Paiva Trugilo, and Karen Brajão de Oliveira *

Laboratory of Molecular Genetics and Immunology, Department of Pathological Sciences, Londrina State University, Londrina, PR, Brazil

* **Correspondence:** Email: karen.brajao@gmail.com; Tel: +55-43-337-15728

Abstract: Human Papillomavirus (HPV) is a necessary cause of cervical cancer in women worldwide. However, the HPV infection is not sufficient to cause neoplasia, and immune mediators, such as chemokines, are important in this context, since they are involved in the regulation of leukocyte trafficking in many essential biological processes, including inflammation. Prolonged inflammation is thought to facilitate carcinogenesis by providing a microenvironment that is ideal for tumor cell development and growth. Chemokines also contribute to tumor development by promoting angiogenesis and metastasis. Among these molecules we highlight the chemokine CXCL12, also called stromal-derived factor 1 alpha (SDF1-α), a pleiotropic chemokine capable of eliciting multiple signal transduction cascades and functions, via interaction with either CXCR4 or CXCR7, which have been implicated in malignant cell survival, proliferation and migration. This review will focus on our current knowledge in the pathogenesis of HPV infection, the main aspects of CXCL12 signaling, its participation in tumor development and immunodeficiencies that may enable the HPV infection. We also discuss how *CXCL12* gene expression and polymorphisms may influence tumor development, especially cervical cancer. Finally, we highlight how the inhibition of CXCL12 pathway may be an attractive alternative for cancer therapeutics.

Keywords: CXCL12; polymorphism; HPV; cervical cancer

Abbreviations

3'UTR 3'	Untranslated Region
ACKR3	Atypical Chemokine Receptor 3
AIDS	Acquired immunodeficiency syndrome
AIP4	Atrophin interacting protein 4
AKT	Protein kinase B
APC	Antigen presenting cell
ASCUS	Atypical Squamous Cells of Undetermined Significance
Bcl-2	B cell lymphoma 2
bFGF	Basic fibroblast growth factor
BMDC	Bone marrow-derived cells
CAF	Lymphocytes, cancer-associated fibroblasts
cAMP	Cyclic adenosine monophosphate
CCL2	Chemokine ligand (family CC) 2 C
CCL5	Chemokine ligand (family CC) 5
CCL7	Chemokine ligand (family CC) 7
CCL8	Chemokine ligand (family CC) 8
CCL20	Chemokine ligand (Family) 20
CCR2	Chemokine receptor (family CC) 2
CCR6	Chemokine receptor (family CC) 6
CIN	Cervical intraepithelial neoplasia
CSCC	Cervical squamous cell carcinoma
CXCL5	Chemokine ligand (family CXC) 5
CXCL8	Chemokine ligand (family CXC) 8
CXCL11	Chemokine ligand (family CXC) 11
CXCL12	Chemokine ligand (family CXC) 12
CXCR4	Chemokine receptor (family CXC) 4
CXCR7	Chemokine receptor (family CXC) 7
DAG	Diacylglycerol
DNA	Desoxyribonucleic acid
E	Early
E2F	E2 promoter-binding factor
E6AP	E6-associated protein
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
ERK1/2	Extracellular signal-regulated kinases ½
GAG	Glycosaminoglycan
GATA 2	GATA binding protein 2
G-CSF	Colony-stimulating factor
GDP	Guanosine diphosphate
GEF	Exchange factor of guanine nucleotide

STAT	Signal transducer and activator of transcription
TAM	Tumor-associated macrophages
TAN	Tumor-associated neutrophils
TCR	T cell receptor
USP14	Ubiquitin-specific protease 14
VEGF	Vascular endothelial growth factor
WHIM	Warts, hypogammaglobulinemia, infections, myelokathexis
ZT	Transformation zone

1. Introduction

Cervical carcinoma is considered an important public health issue. It is the third most common type of cancer in Brazilian women [1] and the fourth in women worldwide [2]. The disease is strongly associated to Human Papillomavirus (HPV) infection, which is present in 99.7% of the cancer cases [3]. There are more than 200 types of the virus and they are classified according to their carcinogenic potential as high-risk, undetermined risk and low-risk [4]. The majority of invasive cervical cancer is associated with HPV16 and 18 [5], both types discovered by Harald Zur Hausen in the 1980s. HPV is also responsible for other types of cancers, such as vulva [6], vagina [7], anus [8], and oropharynx [9], as well as benign diseases such as genital warts and recurrent respiratory papillomatosis [10].

2. HPV Infection

Human Papillomavirus (HPV) is a small non-enveloped desoxyribonucleic acid (DNA) virus that belongs to *Papillomaviridae* family. The doubled-stranded DNA has around 8000bp and consists of eight open reading frames (ORF's) and one non-coding region named long control region (LCR). The ORF's contain seven genes called Early genes (E) and two Late genes (L). Early genes are responsible for the viral replication and late genes for encoding the proteic capsid (Figure 1-A) [11].

The virus may be in episomal state or integrated in the cell genome. In episomal state, high levels of HPV E2 protein suppress the E6 and E7 protein expression. Viral DNA integration usually disrupts E2 expression, leading to the deregulated expression of early viral genes, including E6 and E7, as well as increased proliferative capacity, a crucial step in progression to cancer (Figure 1-B) [12].

E4 protein participation is not fully understood and it is expressed primarily during the late stages of infection, at or around the time that genome amplification begins. It appears to be involved in new virus release and transmission [13]. Many types of HPV show a conserved E5 gene. It appears to play a significant role in virus cell cycle life and is still coevolved with the major HPV oncogenes E6 and E7 [13]. Both proteins, E6 and E7, are strongly expressed in HPV-carrying anogenital malignant tumors [11,14]. E7 protein binds to retinoblastoma protein (pRb), which is a cell cycle down-regulator. Once at its hypophosphorylated state, pRb releases the E2

promoter-binding factor (E2F) at the nucleus, enabling the entry into the S phase of the cell cycle. E7 involvement in the cell cycle is reinforced by E6 protein activity. E6 interacts with E6AP, a cellular ubiquitin ligase, and directs the ubiquitination activity of E6AP especially toward tumor suppressor protein 53 (p53), an important molecule for apoptotic control, driving it to degradation by proteasome [15]. Furthermore, E6AP can also function as a ligand-activated coactivator for the estrogen receptor (ER). The phosphorylated ER binds to E6AP and then, the formed complex is recruited to activate the transcription of a subset of ER target gene promoters [16]. There is strong evidence that estrogen contributes to cervical carcinogenesis through its nuclear ERα. This hormone-receptor axis is required for cervical cancer genesis and persistence, since estrogen seems to be able to increase the expression of the E6 and E7 HPV oncogenes [17,18]. In this sense, the participation of E6AP as ER coactivator and in p53 proteolysis, suggests that the estrogen signaling pathway could be crosstalking with HPV carcinogenesis mechanisms mediated by E6AP in the development of cervical cancer.

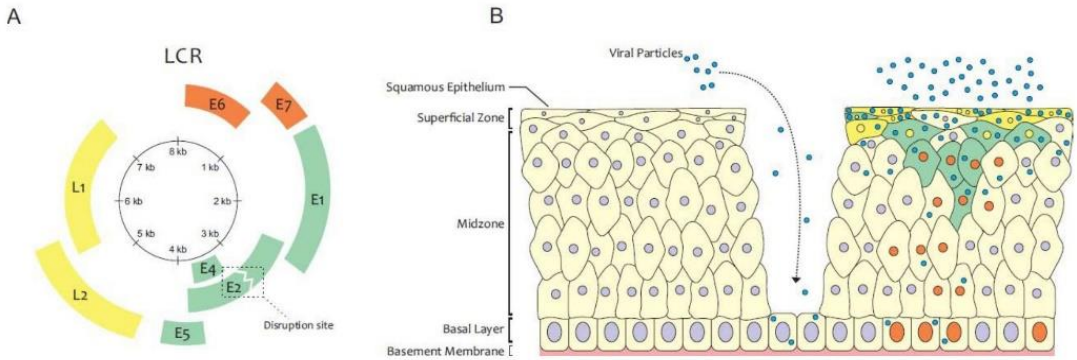

Figure 1. The human papillomavirus genome and the virus life cycle. A) HPV is a double-stranded circular DNA virus. Its genome (about 8000 bp) has 6 early ORFs (E1, E2, E4 and E5 [green]) and E6 and E7 (orange), so called because they are expressed mostly in the early stages of infected keratinocytes differentiation. The late genes L1 and L2 (yellow) code the viral capsid proteins, produced late in the infection. The Long Control Region (LCR) has regulatory elements and transcription factor binding sites that are important in determining and controlling the viral gene expression. The figure depicts the region where viral DNA disruption occurs when HPV integrates into the host genome, enabling cell transformation by overexpression of E6 and E7 oncoproteins. **B**) The HPV is an epitheliotropic and mucosotropic virus that infects basal cells that were exposed by a microwound. Early genes are expressed and viral DNA is productively replicated from episomal DNA. In the upper layers, the late genes are expressed and viral particles are assembled and released. Notice that the viral genes in figure B in each phase of expression are colored according to the genes shown in Figure A.

HPV is transmitted mainly by skin-to-skin or mucosa-to-mucosa contact. Different types of HPV may be transmitted at the same time due to their common route of transmission [17]. Risk factors such as age of the first intercourse, number of sexual partners, condom use, long-term oral contraceptive use, multiparity and smoking are all important for the development of HPV related lesions, but the reason why they function as cofactors is not well established [18].

Most women are infected by at least one type of HPV during their sexual life. The infection may be asymptomatic and may be suppressed by the immunological system in 18 months [18]. Infection occurs through micro wounds in the basal layer and the virus infects cells of the epithelium via a non-established receptor. Infected basal cells migrate to the upper layers of the epithelium and start to differentiate. Then, the virus DNA is packaged forming new capsids and virions are released from the cell [19]. Persistent HPV infection occurs only in a minority of women and evolves to low-grade squamous intraepithelial lesion (LSIL) or high-grade intraepithelial lesion (HSIL) which can still regress or progress to invasive cervical carcinoma [20].

Therefore, infection with high-risk HPV is necessary but not sufficient for cell immortalization and subsequent malignancy. Genetic modifications in the host due to viral DNA integration and chemical and physical mutagens may also contribute to these processes [17]. In addition, exogenous and endogenous factors including tobacco use, parity, oral contraceptive use [20], immune system impairment, and immunological interactions at the site of infection [21] may all influence progression from HPV infection to high-grade cervical lesions.

In this context we highlight the chemokines, since evidence suggests that chemokines are important regulators in the development of viral infections [22] and are also responsible for inducing directional cellular migration, particularly of leukocytes during inflammation, since this prolonged inflammation is thought to facilitate carcinogenesis by providing a microenvironment that is ideal for tumor cell development and growth [23].

3. Chemokines and Cancer Microenvironment

The term chemokines, a short form of “chemotactic cytokines”, was coined in 1992 at a gathering in the elegant castle of Baden near Vienna, after the International Immunology Meeting in Budapest [24]. Chemokines are small proteins from the immune system, which have chemotactic activity that stimulates the migration of different cell types such as lymphocytes, monocytes, neutrophils, endothelial cells, mesenchymal stem cells, and malignant epithelial cells. They constitute the largest family of cytokines, consisting of approximately 50 endogenous chemokine ligands in humans and mice. Chemokines are divided into four subfamilies based on the position of the first two N-terminal cysteine residues, including the CC, CXC, CX3C, and XC subfamilies [24]. Chemokine receptors constitute the largest branch of the γ subfamily of rhodopsin-like seven-transmembrane receptors. Chemokine receptors are differentially expressed on all leukocytes and can be divided into two groups: G protein-coupled chemokine receptors (GPCR), which signal

by activating pertussis toxin (PTX)-sensitive Gi-type G proteins, and atypical chemokine receptors, which appear to shape chemokine gradients and dampen inflammation by scavenging chemokines in a G protein-independent manner. There are approximately 20 signaling chemokine receptors and 5 nonsignaling chemokine receptors [24,25].

Normal tissues carefully control the production and release of growth-promoting signals that instruct entry into and progression through the cell growth and-division cycle, thereby ensuring a homeostasis of cell number and thus maintenance of normal tissue architecture and function. Cancer cells, by deregulating these signals, become masters of their own destinies [27]. Several studies have shown that chemokines and their receptors are implicated in tumor growth and progression. For example, genetic silencing or pharmacologic inhibition of CXCR7 reduced breast tumor growth in mice. Furthermore, MAPK/ERK signaling pathways are downstream targets of the CXCL12/CXCR7 pathway [28].

Another chemokine-mediated mechanism that contributes to tumorigenesis is angiogenesis, a discrete step that is required to allow tumor propagation and progression and the induction of a tumor vasculature [29]. Like normal tissues, tumors require an adequate supply of oxygen, metabolites and an effective way to remove waste products. These requirements vary, however, among tumor types, and change over the course of tumor progression. But gaining access to the host vascular system and the generation of a tumor blood supply are rate-limiting steps in tumor progression [29]. In this way, chemokines and their receptors have been demonstrated as mediators of the vasculogenic process. CXCR4 is expressed in developing vascular endothelial cells and mice lacking CXCR4 or CXCL12 have defective formation of the large vessels that supply the gastrointestinal tract [30], supporting the idea that CXCL12 is a crucial chemokine for the development of new blood vessels. Investigations have demonstrated that low constitutive levels of CXCR4 expression by endothelial cells can be up-regulated by at least 4-fold by the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), rendering endothelial cells more responsive to CXCL12 [31,32].

Tumor progression towards metastasis is often depicted as a multistage process in which malignant cells spread from the original tumor to colonize distant organs. The classical simplification of metastasis into an orderly sequence of basic—local invasion, intravasation, survival in the circulation, extravasation and colonization steps—has helped to rationalize the complex set of biological properties that must be acquired for a particular malignancy to progress towards overt metastatic disease [33]. Not only can tumor cells travel around the body, but they do so under the influence of signals that determine their migratory behavior. A successful metastasis at a new destination requires two stages: first, the cells must respond to chemotactic signals that lead them to “hospitable ground”; and second, they must survive and thrive upon arrival [34]. Chemokines are likely to participate in both processes. Initial evidence that pointed to a role for chemokines in metastasis came from the observation that the expression of chemokine receptors by tumor cells is not random; that is, tumor cells only express selected chemokine receptors [34]. The CXCL12/CXCR4 axis is strongly involved in metastasis. In vivo, neutralizing the interactions of

CXCL12/CXCR4 significantly impairs metastasis of breast cancer cells to regional lymph nodes and the lungs [35]. Malignant melanoma, which has a similar metastatic pattern to breast cancer but also a high incidence of skin metastases, shows high expression levels of CCR10 in addition to CXCR4 and CCR7 [35]. Using CXCR4 antagonists or CXCL12-specific blocking antibodies, many studies have shown these observations in a variety of cancer models, including models of breast cancer, prostate cancer, lung cancer, colorectal cancer, gastric cancer, and glioblastoma [35]. In glioblastoma, CXCL12 has been reported to have direct growth effects mediated through CXCR4. The involvement of CXCR4 in glioblastoma is another example of tumor cells hijacking physiological processes because the CXCL12-CXCR4 axis is important for CNS development and both CXCL12 and CXCR4 are highly expressed in the CNS [36].

Chemokines are emerging as key mediators not only in tumor growth enhancement, blood vessel formation and metastasis, but also in the recruitment of a number of different cell types to the tumor microenvironment. This includes infiltrating cells such as tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs) and lymphocytes, cancer-associated fibroblasts (CAFs), mesenchymal stem cells (MSCs) and endothelial cells [37]. In ovarian cancer, for example, high levels of CXCL12 produced by tumor cells can de-regulate immunity by attracting dendritic cell precursor-2 (preDC2), which does not appear to mediate effective anti-tumor activity, and by altering preDC1 type distribution, immunity and fibrosis stimulation. The result is lack of dendritic cell maturation and antigen presentation failure [37,38].

4. CXCL12 Signaling Pathways

CXCL12 is an important α-chemokine that binds to the G-protein-coupled seven-transmembrane receptor CXCR4. For many years, it was believed that CXCR4 was the only receptor for CXCL12. However, several reports recently provided evidence that CXCL12 also binds to another seven-transmembrane receptor called CXCR7, sharing this receptor with another chemokine family, CXCL11 [39].

The CXCL12-CXCR4 axis may activate many downstream signaling cascades beginning through heterotrimeric G proteins as well as β-arrestins (Figure 2) [40].

Once activated, G protein inhibits adenyl cyclases and cAMP production and stimulates the activity of the Src family tyrosine kinases that activate the Ras/Raf/MEK/ERK pathway, modulating cell cycle progression through the phosphorylation of the adaptor protein Shc. In parallel, CXCR4-oriented migration is mediated by the phosphatidylinositide 3-kinases (PI3Ks). PI3Ks regulate gene transcription, cell migration and cell adhesion by phosphorylating AKT and several focal adhesion components. Furthermore, phospholipase C (PLC) is activated which, in turn, catalyzes phosphatidylinositol 4,5-bisphosphate (PIP2) hydrolysis into inositol 1,4,5-trisphosphate (IP3) and dyacilglycerol (DAG). IP3 production results in Ca^{2+} mobilization from the intracellular stores, while DAG promotes the activation of protein kinase C (PKC) and mitogen associated protein

kinase (MAPK) [26]. CXCL12/CXCR4 also can activate cell proliferation via modulating the Wnt canonical pathway, and suppress apoptosis by NF-κB activation [41,42].

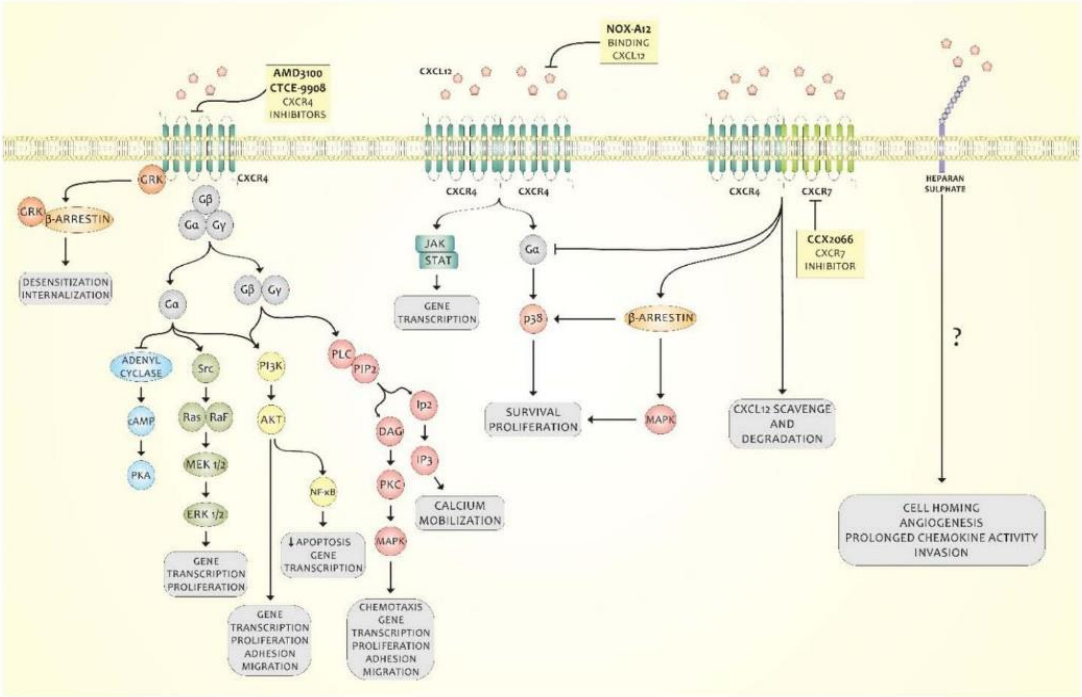

Figure 2. CXCL12/CXCR4/CXCR7 Signaling Pathway. CXCL12 binding to CXCR4, which may form homodimer, triggers G-protein-coupled signaling and subsequent activation of the PI3K/AKT, PLC/IP3, ERK1/2 pathways, resulting in gene transcription, cell adhesion, migration, proliferation, and cell survival. The β-arrestin pathway can be activated through GRK, required for CXCR4 internalization. CXCR4 oligomerization can also activate a G-protein independent pathway via JAK/STAT, inducing gene transcription; p38 may also be activated modulating survival and proliferation. CXCL12 binding to CXCR4-CXCR7 heterodimers can inhibit Gα signaling and potentiates the β-arrestin-dependent downstream signaling, activating p38 and MAPK to increase cell survival. In the latter case, CXCR7 changes the conformation of the CXCR4/G-protein complexes and abrogates signaling. In addition, activation of the β-arrestin pathway may lead to scavenging and degradation of CXCL12. Binding of CXCL12 to heparan sulfate present on cell surface and extracellular matrix prevents its proteolysis and mediates events such as migration, angiogenesis and cancer invasion. The figure depicts drugs tested in clinical and preclinical studies capable of blocking the CXCL12 pathway through receptor inhibition or ligand binding.

CXCR4 activation and phosphorylation can also lead to a dynamic ubiquitination/deubiquitination cycle. According to Bhandari et al. [43], the ubiquitination mechanism is mediated through recruitment of the E3 ubiquitin ligase atrophin interacting protein 4

(AIP4), after CXCR4 activation. A member of the Nedd4-like homologous to E6AP carboxy terminus (HECT) domain family of E3 ubiquitin ligases, AIP4 interacts directly with the C-tail of CXCR4 and ubiquitinates nearby lysine residues, enabling the receptor to be targeted for lysosomal degradation and resulting in CXCR4 down-regulation. While ligand-dependent CXCR4 ubiquitination accelerates CXCR4 degradation, mechanisms for receptor deubiquitination are also activated. CXCL12-CXCR4 binding induces a time-dependent association of CXCR4, or at least its C terminus domain, with ubiquitin-specific protease 14 (USP14), a member of the deubiquitination catalytic family, which deubiquitinates this receptor [44]. These mechanisms seem to be important factors in the regulation of CXCR4 membrane expression and consequently in CXCL12 signaling.

The process of homologous desensitization, or becoming refractory to continued stimulation, is initiated by G protein- coupled receptor kinase (GRK) phosphorylation of serine/threonine residues of the third intracellular loop (TIL) or cytoplasmic tail (C-tail) following receptor activation. This phosphorylation allows for the subsequent binding of arrestin-2 and/or arrestin-3, effectively uncoupling the receptor from further G protein activation and often targeting the receptor for internalization [45].

Moreover, CXCR4 can trigger a G-protein independent signal pathway through association with β-arrestins [46]. It has been reported that arrestin-2 and -3 enhance CXCR4-mediated ERK activation and arrestin-3 is involved in p38 activation and migration following CXCL12 stimulation, thus promoting cell migration [45]. CXCR4 oligomerization has been postulated to play a role in modulating GPCR signaling. Both CXCR4 homodimers and heterodimers have been reported. Homodimerization of CXCR4 has been suggested to result in G-protein-independent signaling through the JAK/Stat signaling pathway [47,48].

In addition to binding to CXCR4, CXCL12 binds to the non-canonical CXCR7 receptor, also known as ACKR3. Depending on the context, CXCL12-CXCR7 binding is thought to result in two different responses: ligand internalization followed by ligand degradation (chemokine clearance), and β-arrestin-mediated G protein-independent signaling. The chemokine clearance function is supported since CXCR7 does not associate with heterotrimeric G proteins, and ligand binding does not evoke calcium influx. The GPCR amino acid sequence motif, DRYLAIV, which is located in the second intracellular loop and is crucial for $G_{\alpha i}$ protein coupling and calcium signaling, is notably lacking in CXCR7. Instead, CXCR7 harbors a DRYLSIT motif, which strongly suggests that this structural difference contributes to CXCR7's inability to relay intracellular signals through $G_{\alpha i}$ [49]. In addition, CXCR7 binds CXCL12 with an affinity ten times higher than CXCR4, and the receptor is rapidly recycled back to the plasma membrane upon ligand-induced internalization. These observations suggest that CXCR7 can act as an efficient chemokine sink because it can successfully compete with CXCR4 for access to the ligand. At least two processes involving CXCR7 may modulate CXCL12 bioavailability. CXCR7 can scavenge CXCL12 and mediate ligand-induced internalization and degradation, thus removing it from the extracellular environment, or modulate the balance between monomeric and dimeric forms of CXCL12 [50]. The co-expression of CXCR7 with

CXCR4 in the same cell may result in the sequestration of CXCL12 from the microenvironment rather than its binding to CXCR4. Mechanisms that alter the overall availability, distribution, and gradients of CXCL12 in tumor microenvironments should, in principle, regulate the functions of CXCR4 in tumor growth and metastasis [50].

Data suggest that CXCL12 may be secreted in two forms: monomer or dimer. The two forms may have distinct roles but their respective actions have not yet been fully elucidated. The balance between the two forms is controlled by factors such as basic pH, the presence of multivalent anions and high concentration of glycosaminoglycans (GAGs) at the cell surface which favor formation of the dimeric form [51]. CXCR7 is also a factor that controls the balance of CXCL12 monomers and dimers because of its preference to link to the CXCL12 monomer [52].

CXCR7 has a role in modulating CXCR4 expression and function. Whether CXCR7 activates or impairs CXCR4 effects depends on the final outcome of their crosstalk. Although GPCRs were previously assumed to act as monomers, recent structural and computational modeling studies have revealed that GPCRs such as CXCR4 can form homodimers and heterodimers, either constitutively or upon ligand binding. GPCR dimer structures have been extensively studied through crystal structure analysis, which can be used to predict the dimerization of the receptor that determines its biological activity and pharmacological effects *in vivo* [50,53].

Putative CXCR4/CXCR7 heterodimers have been proposed to be important regulators of ligand-dependent signaling [54].

The formation of functional heterodimers has been demonstrated using bioluminescence resonance energy transfer assays. Dimerization may result in the recruitment of b-arrestin to the CXCR4/CXCR7 complex [50]. However, CXCR7 may also activate G-protein-independent signaling pathways, as β-arrestin pathway, eliciting pERK signaling to modulate cell fate and migration, what was demonstrated in several systems [50].

Taken together these findings suggest atypical signal properties in CXCR7 leading to cell growth and survival in the presence of CXCL12. The molecular mechanisms which govern the responses are unclear and distinct from common chemokine receptor-mediated signaling [55].

Thus, with CXCR7 identified as a new receptor for CXCL12, the role of the CXCL12-CXCR4 axis in regulating several biological processes became more complex.

Another level of complexity is added by the fact that chemokine function, transport, clearance and degradation could be affected through chemokine binding to GAG, which is present on all animal cell surfaces and in the extracellular matrix [56]. GAGs, in particular heparan sulfate (HS), play a fundamental role in forming the chemokine concentration gradients to establish directional signals for migrating cells. Electrostatic contacts between HS and chemokines determine both the affinity and the specificity of the molecular interactions that are supposed to modulate the *in vivo* biological activity of chemokines complexed to this proteoglycan. It has been shown that the high affinity association of HS with CXCL12, besides locally concentrating the chemokine, prevents its proteolysis [57,58], thus contributing to the increased stability and prolonged immobilization and

activity of CXCL12 in tissues, which can enhance directional migration and tissue homing of cells [57]. Thereby, binding with GAGs seems to regulate important events in both homeostatic and pathologic conditions, such as angiogenesis [57] and cancer invasion [59].

5. *CXCL12* Gene and Cancer

The *CXCL12* gene is located on chromosome 10q.11.1 which was mapped by fluorescence *in situ* hybridization that differs from the location of loci of other known chemokine family members' genes that had been distinguished by chromosomal localizations: genes encoding members of the CC and CXC subfamilies are located on chromosome 17q and 4q, respectively [60]. It was first cloned from a bone marrow-derived stromal cell line and was later identified as a pre-B-cell growth stimulating factor (PBSF). The *CXCL12* gene may have a single nucleotide polymorphism (SNP) in the 3'UTR (3'-untranslated region) also known as rs1801157 (CXCL12 801 G → A) described for the first time in 1998 by Cheryl Winkler [61] who studied patients with AIDS. *CXCL12* polymorphism rs1801157 is also associated to coronary heart disease [62], a possible late-stage protective effect on HIV-1 disease progression in the Brazilian population [63], and as mentioned before, elevated risk of many types of cancer development including breast, lung and lymphoma [64]. Significant frequency of rs1801157 *CXCL12* polymorphism AA genotype was observed in patients with renal cell carcinoma and the overall survival was shorter compared to patients presenting GG and GA genotypes [65]. According to Shimanski et al. [66], patients presenting AA and GA genotypes are more susceptible to distant metastasis development in esophagogastric cancer.

The biological role of *CXCL12* polymorphism in cancer prognosis is controversial. According to Razmkhah et al. [67], this polymorphism is associated with an increased susceptibility to breast cancer development. Combination of low levels of the chemokine and its polymorphism may identify patients with an intrinsic susceptibility to poor survival [68]. In line with this, CXCL12 level measurement may be an important biomarker to cancer prognosis. Although CXCL12 mRNA has been reported to be expressed in many different tissues, quantitative analysis of CXCL12 expression in many human organs of patients with breast cancer revealed that CXCL12 mRNA is expressed preferentially in lymph nodes, the lungs, liver and bone marrow. Phillips et al. [69], using an animal model of xenoengraftment of human non-small cell lung cancer (NSCLC) cells, found that CXCL12 protein levels are significantly higher in the adrenal glands, the lungs, liver and bone marrow, that are known to be highly susceptible to human NSCLC metastasis. Moreover, the plasmatic level of CXCL12 was increased in patients with prostate cancer compared to those who had a benign form of the disease and health controls [70] and in tumoral bone marrow cells in multiple myeloma [71].

Furthermore, Sei et al. [72] demonstrated that CXCL12 mRNA may be up-regulated due to the gene variation located within 3' UTR suggesting that *CXCL12* polymorphism may influence the abundance of the chemokine. However, de Oliveira et al. [73] observed that allele A breast cancer

patients presented a mRNA CXCL12 expression about 2.1-fold smaller than GG breast cancer patients, suggesting that allele A is associated with low expression of CXCL12 in the peripheral blood from ER-positive breast cancer patients.

Little is known about the chemokine CXCL12 and its polymorphisms in cervical cancer and the published data so far are controversial.

6. Involvement of CXCL12 Axis in HPV Infection Susceptibility

Host defense against HPV relies on intact and functioning cellular immunity, including T-cell and natural killer (NK) cell cytotoxicity. Patients in whom warts are severe or recalcitrant, concern for immune defects is raised [74].

The WHIM syndrome features susceptibility to human Papillomavirus infection-induced warts and carcinomas, hypogammaglobulinemia, recurrent bacterial infections, B and T-cell lymphopenia, and neutropenia associated with retention of senescent neutrophils in the bone marrow (i.e. myelokathexis). WHIM syndrome patients present abnormal expression of *CXCL12* and its receptors in keratinocytes immortalized by HPV16 and 18 in a dependent way of E6 and E7 oncoprotein expression. E6 and E7 proteins expressed in the suprabasal layer would positively regulate CXCL12 levels and its receptor, which would raise cell proliferation and viral replication. This phenomenon may occur for low and high risk HPV and could be enhanced by *CXCR4* mutation that may provide a base for development of WHIM verrucose patients and HPV associated to oncogenesis [75]. A key marker seen in the WHIM syndrome is the increased activation of the CXCL12/CXCR4 axis, which usually results from gain-of-function mutations in *CXCR4.* CXCR4 mutants maintain association with β-arrestins and trigger abnormal β-arrestin-dependent pathways as revealed by activation of the ERK1/2 signaling. Although the immuno-hematological manifestations of the WHIM syndrome apparently result from CXCR4 dysfunctions, the mechanisms by which these dysfunctions might affect leukocyte homeostasis remain unknown [76].

GATA2 deficiency is associated with impaired membrane expression and chemotactic dysfunctions of CXCR4. Patients suffering from GATA2 deficiency also display a high susceptibility to HPV infections, as do patients with the WHIM syndrome. CXCR4-dependent chemotactic responses in patients with GATA2 deficiency are differently affected among lymphocytes with a marked loss of function for NK cells but an enhanced function for B lymphocytes. These dysfunctions may contribute to the physiopathology of this deficiency by affecting the normal distribution of lymphocytes and thus potentially affecting the susceptibility of patients to associated infections [77].

In Idiopathic CD4 lymphopenia (ICL) cryptococcosis, HPV, and nontuberculous mycobacterial infection were the three most common infections. The couple CXCL12/CXCR4 directs thymopoiesis and regulates the domiciliation of lymphocyte T (LT) in secondary lymphoid organs. Loss or gain of CXCR4 expression in mice leads to a decrease in LT CD^{4+} associated with a defect in thymic

maturation and exacerbates migration to the bone marrow [78]. Warts in patients with ICL are typically presented as disseminated verrucae or flat warts on the extremities, face, and genitals. A few reports have found HPV types 2, 3, 6, and 49 in cutaneous lesions. HPV-related dysplasia and carcinoma can be the presentation of ICL [74]. These results underscore the importance of delineating the mechanisms underlying CXCL12/CXCR4 dysfunctions, including their penetrance and potential role in the pathogenesis of rare deficiencies. Host defense against HPV is multifaceted. Investigation of patients with severe recalcitrant HPV has increased our understanding of host defense and cutaneous and systemic immunity [74,77].

7. CXCL12 and Cervical Cancer

Five polymorphisms of the *CXCL12* gene were analyzed in a based-population case-control study where women with invasive squamous carcinoma and adenocarcinoma *in situ* were investigated. The authors concluded that *CXCL12* polymorphism rs1801157 was not associated with cervical cancer risk [79]. According to Tee et al. [80] the same polymorphism raises no susceptibility to neoplastic lesions of the uterine cervix. No significant difference of genotype frequencies of the *CXCL12* polymorphism was found between patients and controls. However, a stratified analysis between the *CXCL12* rs1801157 genotype distribution and cervical cancer risk showed that the polymorphism may be a risk factor for patients with a positive history of tobacco smoking [81].

A few studies were performed in order to elucidate the transcriptional pattern of the *CXCL12* gene in cervical cancer. Jaafar et al. [82] have shown overexpression of *CXCL12* in squamous and glandular cervical lesions. This increased expression was associated with infiltration of $FOXP3^{+}$ regulatory T cells (known to be associated with cervical cancer development), cancer staging, histopathological progression and HPV infection, indicating that CXCL12 could be a good marker for clinical progression. Also, Huang et al. [83] demonstrated that the expression of CXCL12 and its receptor CXCR4 was significantly higher in the cervical cancer group than in the Cervical intraepithelial neoplasia CIN1 and CIN2 groups. CXCR7 was also higher expressed in cervical cancer than in CIN and normal cervical mucosa, especially in those patients with advanced staging and lymph node metastasis. CXCL12 appeared to be positively regulated by CXCR7 at the post-transcriptional level in CSCC (cervical squamous cell carcinoma) [84]. Zanotta et al. [85] studied the soluble immune mediators in young women with HR-HPV (High Risk HPV). Data showed that CXCL12 was significantly linked with HR-HPV infection and increased levels have been detected in women with pre-cancerous lesion.

Correlation between overexpression of CXCL12/CXCR4 and lymph node metastasis was also observed by Wei et al. [86]. A variable number of tumor cells in 33 specimens expressed CXCL12. The overexpression rate of CXCL12 was significantly higher in IB cases than in IIB cases or in tumors with lymph node metastasis. The exact mechanism of CXCL12 and its receptor CXCR4 in cervical cancer metastasis development still needs to be fully elucidated.

In order to investigate the role of the CXCL12/CXCR4 axis in mediating metastasis in cervical cancer cells, Shen et al. [87] analyzed the phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 in HeLa cells after binding CXCL12 to CXCR4 and observed that, in HeLa cells exposed to CXCL12, ERK-1/2 was rapidly phosphorylated, that the adhesion ability of HeLa cells to fibronectin and laminin was increased after CXCL12 treatment while pretreatment of HeLa cells with an ERK-1/2 inhibitor, PD98059, decreased adhesion of HeLa cells to the extracellular matrix with the presence of CXCL12. They also observed that increased amounts of active matrix metalloproteinase 2 (MMP2) were secreted in response to increased CXCL12 concentrations, suggesting therefore that CXCL12/CXCR4 participates in tumor invasiveness and metastasis in cervical cancer through regulating the adhesion ability by activating the MAPK signaling transduction pathway and promoting MMP2 secretion. The events discussed above are summarized in Figure 3.

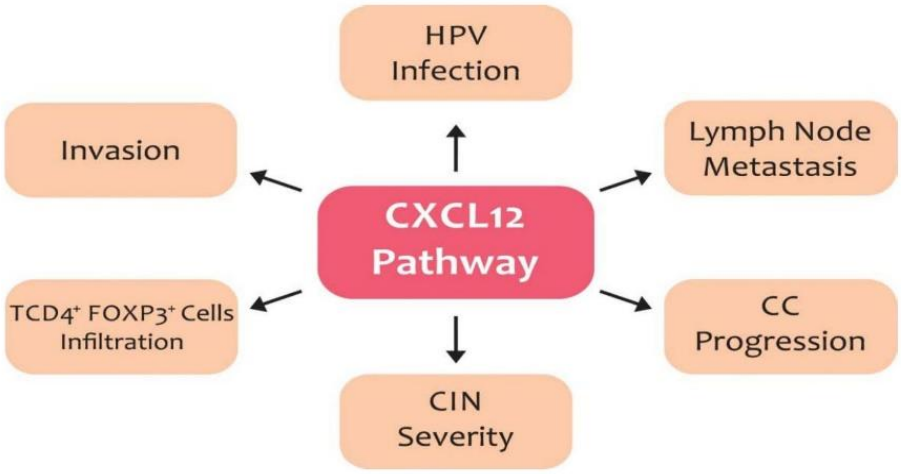

Figure 3. Events mediated by CXCL12 pathway in HPV infection and carcinogenesis. HPV: human papillomavirus; CC: cervical cancer; CIN: cervical intraepithelial neoplasia.

Yadav et al. [88] investigated whether deregulation in CXCR4 signaling (as a consequence of deregulated CXCL12 expression) modulates the metastatic potential of cervical carcinoma cells, and observed a controversial result suggesting the tumor suppressor functions of CXCL12 in cervical cancer. They demonstrated that CXCL12 is frequently downregulated and its promoter is hypermethylated in cervical cancer cell lines and primary tumor biopsies. This suggests that (a) silencing of CXCL12 in cervical cancer cells may be critical in migration and invasion, the key events in cancer cell metastases; (b) cervical cancer cells having downregulated CXCL12 are more prone to being attracted to CXCL12 expressed at secondary sites of metastases; and (c) CXCL12 inhibits anchorage independent cell growth via anoikis.

Since CXCL12, through its receptors, CXCR4 and CXCR7, plays critical roles in mediating tumor metastasis and angiogenesis in several types of cancers including cervical cancer, the CXCL12/CXCR4/CXCR7 axis is a potential target for therapeutics that block the CXCL12/CXCR4 or CXCL12/CXCR7 interactions or which inhibit activities of downstream signaling.

8. CXCL12/CXCR4/CXCR7 Blockade

Considering the significance of the CXCL12 pathway in cancer development, some drugs were developed to impair this axis and showed promising results. These drugs comprise AMD3100, or plerixafor (Mozobil; Sanofi SA, Paris, France), a CXCR4 antagonist; CTCE-9908 (Chemokine Therapeutics Corp, Vancouver, BC, Canada), a CXCL12 analog; Nox-A12 (Noxxon Pharma AG, Berlin, Germany), an anti-CXCL12 aptamer; and CCX2066 (ChemoCentryx, Inc), a CXCR7-specific inhibitor [89–91]. There are reports using interference RNA, that might be a potential therapy tool [92–94]. Of these, the use has been clinically approved [90,95] of AMD3100 for stem cell mobilization in patients with non-Hodgkin's lymphoma and multiple myeloma and CTCE-9908 for patients with osteosarcoma. AMD3100 is the better studied drug among agents that antagonize CXCR4. Initially researched as an anti-HIV drug, AMD3100 has already been shown to decrease metastatic potential in animal models for different types of tumors, including breast, ovarian, colorectal cancer and melanoma [46]. In an HPV-induced disease context, Uchida et al. [95] observed that treatment with AMD3100 inhibited lymph node metastases of the oral squamous cell carcinoma cell line (B88) when they were orthotopically inoculated into nude mice. It is known that oral squamous cell carcinoma is highly associated with HPV infection [96]. Furthermore, in a transgenic mouse model of HPV-induced epidermal neoplasia, daily treatment with AMD3100 abrogated CXCL12 and cyclin-dependent kinase inhibitor p16INK4a coexpression in dysplastic epidermis [97]. p16INK4a is considered a marker of virus-induced cell cycle deregulation [98]. The same study also reported reduced papilloma development on the ears and reduced ear thickness of the treated mice, chronic inflammation impairment in the course of premalignant progression and decreased proliferation and increased apoptosis of keratinocytes [97]. Administration of AMD3100 also is a clinical indication for the WHIM syndrome [26].

Studies on the blockade of the CXCL12 pathway differ and must be carefully analyzed. Experimental studies have shown that anti-CXCL12 drugs can be effective in decreasing tumor growth and metastasis in human and animal models at the beginning of treatment or tumor establishment, but low efficiency in established tumors [99–101], although tumor growth was minor in brain and prostatic human cancers [102,103]. Otherwise, the CXCL12 blockade might be more useful in metastasis prevention. Dose-dense adjuvant chemotherapy is given to women with breast cancer together with granulocyte colony-stimulating factor (G-CSF), and G-CSF may induce proteolytic disruption of the CXCL12/CXCR4 axis, inhibiting CXCR4-dependent homing micrometastatics [104]. Furthermore, clinical and preclinical studies demonstrated that treatment

with some antiangiogenic (e.g.: cediranib), cytotoxic (e.g.: paclitaxel) and vascular-disrupting agents (e.g.: OXi-4503) drugs may increase circulating CXCL12 and bone marrow-derived cell (BMDC) infiltration, thus favoring angiogenesis and metastasis [105–108]. Moreover, concomitant therapy of anti-CXCR4 AMD3100 and radiotherapy delayed tumor growth and increased tumor clearance in brain, lung and breast cancers in xenograft models [109,110]. Some clinical studies also showed high CXCL12, CXCR4 and CXCR7 expression in advanced gliobastoma [86,111,112] and elevated circulating CXCL12 after treatment with VEGFR tyrosine kinase inhibitor cediranib [105,113]. These data suggest that the CXCL12 blockade in combination with other therapies might prevent tumor recurrence and decrease the chemoresistance-induced CXCL12 and further, induce tumor sensitization to other therapies.

9. Concluding Remarks

Taken together, these data support the fact that the CXCL12 pathway is a potential target for the emerging therapies that aim to delay cancer progression, angiogenesis and metastasis, since they may act synergistically with the adjuvant and neoadjuvant anti-tumor treatment. Since data have shown that CXCL12 mediates not only cervical cancer cell migration but also influences overall cancer prognosis, the understanding of tumor biology with focus on CXCL12 will enable the success of therapy currently used and the design of new therapeutic interventions.

Conflict of Interest

All the authors declare no conflicts of interest in this paper.

References

1. BRASIL. Estimativa 2016: Incidência de Câncer no Brasil. *INCA* 2015.
2. Torre LA, Bray F, Siegel RL, et al. (2015) Global Cancer Statistics, 2012. *CA a cancer J Clin* 65: 87-108.
3. Walboomers JMM, Jacobs MV, Manos MM, et al. (1999) Human Papillomavirus Is a Necessary Cause Of Invasive Cervical Cancer Worldwide. *J Patholgy* 189: 12-19.
4. Woodman CBJ, Collins SI, Young LS (2007) The natural history of cervical HPV infection: unresolved issues. *Nat Rev Cancer* 7: 11-22.
5. Smith JS, Lindsay L, Hoots B, et al. (2007) Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions A meta -analysis update. *Int J Cancer* 121: 621-632.
6. Lee LJ, Howitt B, Catalano P, et al. (2016) Gynecologic Oncology Prognostic importance of human papillomavirus (HPV) and p16 positivity in squamous cell carcinoma of the vulva treated

with radiotherapy. *Gynecol Oncol* 142: 293-298.

7. Levovitz C, Chen D, Ivansson E, et al. (2014) TGFβ Receptor 1: An immune susceptibility gene in HPV-associated cancer. *Cancer Res* 74: 6833-6844.
8. Mai S, Welzel G, Ottstadt M, et al. (2015) Prognostic relevance of HPV infection and p16 overexpression in squamous cell anal cancer. *Radiat Oncol Biol* 93: 819-827.
9. Egawa N, Egawa K, Griffin H, et al. (2015) Human Papillomaviruses; epithelial tropisms, and the development of neoplasia. *Viruses* 7: 3863-3890.
10. Arbyn M, Sanjosé S de, Saraiya M, et al. (2011) EUROGIN 2011 roadmap on prevention and treatment of HPV- related disease. *Int J Cancer* 131: 1969-1982.
11. Doorbar J (2006) Molecular biology of human papillomavirus infection and cervical cancer. *Clin Sci* 110: 525-541.
12. Moody CA, Laimins LA (2010) Human papillomavirus oncoproteins pathways to transformation. *Nat Rev Cancer* 10: 550-560.
13. Doorbar J (2013) The E4 protein —structure , function and patterns of expression. *Virology* 445: 80-98.
14. DiMaio D, Petti LM (2013) The E5 proteins. *Virology* 445: 99-114.
15. Beaudenon S, Huibregtse JM (2008) HPV E6 , E6AP and cervical cancer. *BMC Biochem* 7: 1-7.
16. Zhou W, Slingerland JM (2014) Links between oestrogen receptor activation and proteolysis relevance to hormone-regulated cancer therapy. *Nat Rev Cancer* 14: 26-38.
17. zur Hausen H (2009) Papillomaviruses in the causation of human cancers—a brief historical account. *Virology* 384: 260-265.
18. Schiffman M, Castle PE, Jeronimo J, et al. (2007) Human papillomavirus and cervical cancer. *Lancet* 370: 890-907.
19. Schiffman M, Wentzensen N (2013) Human Papillomavirus Infection and the Multistage Carcinogenesis of Cervical Cancer. *Cancer Epidemiol Biomarkers Prev* 22: 553-560.
20. Castellsagué X, Muñoz N (2003) Chapter 3: Cofactors in human papillomavirus carcinogenesis—role of parity, oral contraceptives, and tobacco smoking. *J Nation Cancer Institute Monographs* 31: 20-28.
21. Patel S, Chiplunkar S (2009) Host immune responses to cervical cancer. *Curr Opin Obstet Gynecol* 21: 54-59.
22. Mbeunkui F, Johann DJJr (2010) Cancer and the tumor microenvironment: a review of an essential relationship. *Cancer Chemoter Pharmacol* 63: 571-582.
23. Vandercappellen J, Van Damme J, Struyf S (2008) The role of CXC chemokines and their receptors in cancer. *Cancer Lett* 267: 226-244.
24. Baggiolini M (2001) Chemokines in pathology and medicine. *J Intern Med* 250: 91-104.
25. Griffith JW, Sokol CL, Luster AD (2014) Chemokines and chemokine receptors: positioning cells for host defense and immunity. *Annu Rev Immunol* 32: 659-702.
26. Pozzobon T, Goldoni G, Viola A, et al. (2016) CXCR4 signaling in health and disease. *Immunol*

Lett 177: 6-15.

27. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144: 646-674.
28. Wani N, Nasser MW, Ahirwar DK, et al. (2014) C-X-C motif chemokine 12/C-X-C chemokine receptor type 7 signaling regulates breast cancer growth and metastasis by modulating the tumor microenvironment. *Breast Cancer Res*16: R54.
29. Bergers G, Benjamin LE (2003) Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 3: 401-410.
30. Tachibana K, Hirota S, Iizasa H, et al. (1998). The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. *Nature* 393: 591-594.
31. Funakoshi A, Jimi A, Yasunami Y, et al. (1989) CXCL8/IL8 stimulates vascular endothelial growth factor (VEGF) expression and the autocrine activation of VEGFR2 in endothelial cells by activating NFkappaB through the CBM (Carma3/Bcl10/Malt1) complex. *Biochem Biophys Res Commun* 159: 913-918.
32. Martin D, Galisteo R, Gutkind JS (2009) CXCL8/IL8 stimulates vascular endothelial growth factor (VEGF) expression and the autocrine activation of VEGFR2 in endothelial cells by activating NF-κB through the CBM (Carma3/Bcl10/Malt1) complex. *J Biol Chem* 284: 6038-6042.
33. Nguyen DX, Bos PD, Massagué J (2009) Metastasis: from dissemination to organ-specific colonization. *Nat Rev Cancer* 9: 274-284.
34. Zlotnik A, Burkhardt AM, Homey B (2011) Homeostatic chemokine receptors and organ-specific metastasis. *Nat Rev Immunol* 11: 597-606.
35. Muller A, Homey B, Soto H, et al. (2001) Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410: 50-56.
36. Terasaki M, Sugita Y, Arakawa F, et al. (2011) CXCL12/CXCR4 signaling in malignant brain tumors: a potential pharmacological therapeutic target. *Brain Tumor Pathol* 28: 89-97.
37. Balkwill F (2004) Cancer and the chemokine network. *Nat Rev Cancer* 4: 540-550.
38. Zou W, Machelon V, Coulomb-L'Hermin A, et al. (2001) Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. *Nat Med* 7: 1339-1346.
39. Maksym RB, Tarnowski M, Grymula K, et al. (2009) The role of stromal derived factor-1-CXCR7 axis in development and cancer. *Eur J Pharmacol* 625: 31-40.
40. Wang J, Knaut H (2014) Chemokine signaling in development and disease. *Development* 12: 4199-4205.
41. Wang Z, Ma Q, Liu Q, et al. (2008) Blockade of SDF-1/CXCR4 signalling inhibits pancreatic cancer progression in vitro via inactivation of canonical Wnt pathway. *Br J Cancer* 99: 1695-1703.
42. Ganju RK, Brubaker SA, Meyer J, et al. (1998) The a-Chemokine, stromal cell-derived factor-1a, binds to the transmembrane G-protein-coupled CXCR-4 receptor and activates multiple signal

transduction pathways. *J Biol Chem* 273: 23169-23175.

43. Bhandari D, Robia SL, Marchese A (2009) The E3 ubiquitin ligase atrophin interacting protein 4 binds directly to the chemokine receptor CXCR4 via a novel WW domain-mediated interaction. *Mol Biol Cell* 20: 1324-1339.
44. Mines MA, Goodwin JS, Limbird LE, et al. (2009) Deubiquitination of CXCR4 by USP14 is critical for both CXCL12-induced CXCR4 degradation and chemotaxis but not ERK activation. *J Biol Chem* 284: 5742-5752.
45. Busillo JM, Benovic JL (2007) Regulation of CXCR4 signaling. *Biochim Biophys Acta* 1768: 952-963.
46. Cojoc M, Peitzsch C, Trautmann F, et al. (2013) Emerging targets in cancer management: role of the CXCL12/CXCR4 axis. *Onco Targets Ther* 6: 1347-1361.
47. Mellado M, Rodríguez-Frade JM, Mañes S, et al. (2001) Chemokine signaling and functional responses: the role of receptor dimerization and TK pathway activation. *Annu Rev Immunol* 19: 397-421.
48. Teicher BA, Fricker SP (2010) CXCL12 (SDF-1)/CXCR4 Pathway in Cancer. *Clin Cancer Res* 16: 2927-2931.
49. Graham GJ, Locati M, Mantovani A, et al. (2012) The biochemistry and biology of the atypical chemokine receptors. *Immunol Lett* 145: 30-38.
50. Freitas C, Desnoyer A, Meuris F, et al. (2014) The relevance of the chemokine receptor ACKR3/CXCR7 on CXCL12-mediated effects in cancers with a focus on virus-related cancers. *Cytokine Growth Factor Rev* 25: 307-316.
51. Veldkamp CT, Peterson FC, Pelzek AJ, et al. (2005) The monomer—dimer equilibrium of stromal cell-derived factor-1 (CXCL 12) is altered by pH , phosphate , sulfate , and heparin. *Protein Sci* 1: 1071-1081.
52. Ray P, Lewin SA, Mihalko LA, et al. (2012) Secreted CXCL12 (SDF-1) forms dimers under physiological conditions. *Biochem J* 442: 433-442.
53. Wu B, Chien EY, Mol CD, et al. (2010) Structures of the CXCR4 Chemokine. *Science* 330: 1066-1071.
54. Kramp BK, Sarabi A, Koenen RR, et al. (2011) Heterophilic chemokine receptor interactions in chemokine signaling and biology. *Exp Cell Res* 317: 655-663.
55. Thelen M, Thelen S (2008) CXCR7, CXCR4 and CXCL12: An eccentric trio? *J Neuroimmunol* 198: 9-13.
56. Laguri C, Arenzana-Seisdedos F, Lortat-Jacob H (2008) Relationships between glycosaminoglycan and receptor binding sites in chemokines-the CXCL12 example. *Carbohydr Res* 343: 2018-2023.
57. Rueda P, Balabanian K, Lagane B, et al. (2008) The CXCL12γ Chemokine Displays Unprecedented Structural and Functional Properties that Make It a Paradigm of Chemoattractant Proteins. Gold JA, editor. *PLoS One* 3: e2543.

58. Sadir R, Imberty A, Baleux F, et al. (2004) Heparan Sulfate/Heparin oligosaccharides protect stromal cell-derived factor-1 (SDF-1)/CXCL12 against proteolysis induced by CD26/dipeptidyl peptidase IV. *J Biol Chem* 279: 43854-43860.
59. Brule S, Friand V, Sutton A, et al. (2009) Glycosaminoglycans and syndecan-4 are involved in SDF-1/CXCL12-mediated invasion of human epitheloid carcinoma HeLa cells. *Biochim Biophys Acta-Gen Subj* 1790: 1643-1650.
60. Shirozu M, Nakano T, Inazawa J, et al. (1995) Struture and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. *Genomics* 28: 495-500.
61. Winkler C, Modi W, Smith MW, et al. (1998) Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. ALIVE Study, Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC). *Science* 279: 389-393.
62. Feng L, Nian S, Hao Y, et al. (2014) A Single Nucleotide Polymorphism in the Stromal Cell-Derived Factor 1 Gene Is Associated with Coronary Heart Disease in Chinese Patients. *Int J Mol Sci* 15: 11054-11063.
63. Reiche EMV, Ehara Watanabe MA, et al. (2006) The effect of stromal cell-derived factor 1 (SDF1/CXCL12) genetic polymorphism on HIV-1 disease progression. *Int J Mol Med* 18 785-793.
64. de Oliveira KB, Oda JMM, Voltarelli JC, et al. (2009) CXCL12 rs1801157 polymorphism in patients with breast cancer, hodgkin's lymphoma, and non-hodgkin's lymphoma. *J Clin Lab Anal* 23: 387-393.
65. Cai C, Wang L-H, Dong Q, et al. (2013) Association of CXCL12 and CXCR4 gene polymorphisms with the susceptibility and prognosis of renal cell carcinoma. *Tissue Antigens* 82: 165-170.
66. Schimanski C, Jordan M, Schlaegel F (2011) SNP rs1801157 significantly correlates with distant metastasis in CXCL12 expressing esophagogastri c cancer. *Int J Oncol* 39: 515-920.
67. Razmkhah M, Doroudchi M, Ghayumi SMA, et al. (2005) Stromal cell-derived factor-1 (SDF-1) gene and susceptibility of Iranian patients with lung cancer. *Lung Cancer* 49: 311-315.
68. Hassan S, Baccarelli A, Salvucci O, et al. (2008) Plasma stromal cell-derived factor-1: host derived marker predictive of distant metastasis in breast cancer. *Clin Cancer Res* 14: 446-454.
69. Phillips RJ, Burdick MD, Lutz M, et al. (2003) The stromal derived factor–1/CXCL12–CXC chemokine receptor 4 biological axis in non–small cell lung cancer metastases. *Am J Respir Crit Care Med* 167: 1676-1686.
70. Dehghani M, Kianpour S, Zangeneh A, et al. (2014) CXCL12 modulates prostate cancer cell adhesion by altering the levels or activities of β1-containing integrins. *Int J Cell Biol* 2014: 981750.
71. Beider K, Bitner H, Leiba M, et al. (2014) Multiple myeloma cells recruit tumor-supportive macrophages through the CXCR4/CXCL12 axis and promote their polarization toward the M2

phenotype. *Oncotarget* 5: 11283-11296.

72. Sei S, O'Neill DP, Stewart SK, et al. (2001) Increased level of stromal cell-derived factor-1 mRNA in peripheral blood mononuclear cells from children with AIDS-related lymphoma. *Cancer Res* 61: 5028-5037.
73. de Oliveira KB, Guembarovski RL, Oda JMM, et al. (2011) CXCL12 rs1801157 polymorphism and expression in peripheral blood from breast cancer patients. *Cytokine* 55: 260-265.
74. Leiding JW, Holland SM (2012) Warts and all: human papillomavirus in primary immunodeficiencies. *J Allergy Clin Immunol* 130: 1030-1048.
75. Chow KYC, Brotin É, Ben Khalifa Y, et al. (2010) A Pivotal Role for CXCL12 Signaling in HPV-Mediated Transformation of Keratinocytes: Clues to Understanding HPV-Pathogenesis in WHIM Syndrome. *Cell Host Microbe* 8: 523-533.
76. Bachelerie F (2010) CXCL12/CXCR4-axis dysfunctions: Markers of the rare immunodeficiency disorder WHIM syndrome. *Dis Markers* 29: 189-198.
77. Maciejewski-Duval A, Meuris F, Bignon A, et al. (2016) Altered chemotactic response to CXCL12 in patients carrying GATA2 mutations. *J Leukoc Biol* 99: 1065-1076.
78. Bignon A, Biajoux V, Bouchet-Delbos L, et al. (2011) CXCR4, a therapeutic target in rare immunodeficiencies? *Med Sci* (Paris) 27: 391-397.
79. Maley SN, Schwartz SM, Johnson LG, et al. (2009) Genetic variation in CXCL12 and risk of cervical carcinoma: a population-based case-control study. *Int J Immunogenet* 36: 367-375.
80. Tee Y, Yang S-F, Wang P, et al. (2012) G801A Polymorphism of Human Stromal Cell–Derived Factor 1 Gene Raises No Susceptibility to Neoplastic Lesions of Uterine Cervix. *Int J Gynecol Cancer* 22: 1297-1302.
81. Roszak A, Misztal M, Sowińska A, et al. (2015) Stromal cell-derived factor-1 G801A polymorphism and the risk factors for cervical cancer. *Mol Med Rep* 11: 4633-4638.
82. Jaafar F, Righi E, Lindstrom V, et al. (2009) Correlation of CXCL12 expression and $FoxP^{3+}$ cell infiltration with human papillomavirus infection and clinicopathological progression of cervical cancer. *Am J Pathol* 175: 1525-1535.
83. Huang Y, Zhang J, Cui Z-M, et al. (2013) Expression of the CXCL12/CXCR4 and CXCL16/CXCR6 axes in cervical intraepithelial neoplasia and cervical cancer. *Chin J Cancer* 32: 289-296.
84. Kurban S, Tursun M, Kurban G, et al. (2014) Role of CXCR7 and effects on CXCL12 in SiHa cells and upregulation in cervical squamous cell carcinomas in Uighur women. *Asian Pac J Cancer Prev* 15: 9211-9216
85. Zanotta N, Tornesello ML, Annunziata C, et al. (2016) Candidate soluble immune mediators in young women with high-risk human papillomavirus infection: high expression of chemokines promoting angiogenesis and cell proliferation. *PLoS One* 11: e0151851.
86. Baker R, Dauner JG, Rodriguez AC, et al. (2011) Increased plasma levels of adipokines and inflammatory markers in older women with persistent HPV infection. *Cytokine* 53: 282-285.

87. Shen X, Wang S-H, Liang M, et al. (2008) The role and mechanism of CXCR4 and its ligand SDF-1 in the development of cervical cancer metastasis. *Ai Zheng* 27: 1044-1049.
88. Yadav SS, Prasad SB, Das M, et al. (2014) Epigenetic silencing of CXCR4 promotes loss of cell adhesion in cervical cancer. *Biomed Res Int* 2014: 581403.
89. Donzella GA, Schols D, Lin SW, et al. (1998) AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co-receptor. *Nat Med* 4: 72-77.
90. Burger JA, Stewart DJ (2009) CXCR4 chemokine receptor antagonists: perspectives in SCLC. *Expert Opin Investig Drugs* 18: 481-490.
91. Sayyed SG, Hägele H, Kulkarni OP, et al. (2009) Podocytes produce homeostatic chemokine stromal cell-derived factor-1/CXCL12, which contributes to glomerulosclerosis, podocyte loss and albuminuria in a mouse model of type 2 diabetes. *Diabetologia* 52: 2445-2454.
92. Miao Z, Luker KE, Summers BC, et al. (2007) CXCR7 (RDC1) promotes breast and lung tumor growth in vivo and is expressed on tumor-associated vasculature. *Proc Natl Acad Sci USA* 104: 15735-15740.
93. Wang J, Wang J, Sun Y, et al. (2005) Diverse signaling pathways through the SDF-1/CXCR4 chemokine axis in prostate cancer cell lines leads to altered patterns of cytokine secretion and angiogenesis. *Cell Signal* 17: 1578-1592.
94. Sowińska A, Jagodzinski PP (2007) RNA interference-mediated knockdown of DNMT1 and DNMT3B induces CXCL12 expression in MCF-7 breast cancer and AsPC1 pancreatic carcinoma cell lines. *Cancer Lett* 255: 153-159.
95. Uchida D, Onoue T, Kuribayashi N, et al. (2011) Blockade of CXCR4 in oral squamous cell carcinoma inhibits lymph node metastases. *Eur J Cancer* 47: 452-459.
96. Gupta S, Gupta S (2015) Role of human papillomavirus in oral squamous cell carcinoma and oral potentially malignant disorders: A review of the literature. *Indian J Dent* 6: 91-98.
97. Meuris F, Gaudin F, Aknin M-L, et al. (2016) Symptomatic Improvement in Human Papillomavirus-Induced Epithelial Neoplasia by Specific Targeting of the CXCR4 Chemokine Receptor. *J Invest Dermatol* 136: 473-480.
98. von Knebel Doeberitz M (2001) New molecular tools for efficient screening of cervical cancer. *Dis Markers* 17: 123-128.
99. Sun Y, Schneider A, Jung Y, et al. (2005) Skeletal localization and neutralization of the SDF-1(CXCL12)/CXCR4 axis blocks prostate cancer metastasis and growth in osseous sites in vivo. *J Bone Miner Res* 20: 318-329.
100. Huang EH, Singh B, Cristofanilli M, et al. (2009) A CXCR4 Antagonist CTCE-9908 Inhibits Primary Tumor Growth and Metastasis of Breast Cancer. *J Surg Res* 155: 231-236.
101. Matsusue R, Kubo H, Hisamori S, et al. (2009) Hepatic stellate Cells cromote liver metastasis of colon cancer cells by the action of SDF-1/CXCR4 axis. *Ann Surg Oncol* 16: 2645-2653.
102. Rubin JB, Kung AL, Klein RS, et al. (2003) A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. *Proc Natl Acad Sci USA* 100: 13513-13518.

103. Porvasnik S, Sakamoto N, Kusmartsev S, et al. (2009) Effects of CXCR4 antagonist CTCE-9908 on prostate tumor growth. *Prostate* 69: 1460-1469.
104. Epstein RJ (2004) The CXCL12-CXCR4 chemotactic pathway as a target of adjuvant breast cancer therapies. *Nat Rev Cancer* 4: 901-909.
105. Batchelor TT, Sorensen AG, di Tomaso E, et al. (2007) AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. *Cancer Cell* 11: 83-95.
106. Kamoun WS, Ley CD, Farrar CT, et al. (2009) Edema control by cediranib, a vascular endothelial growth factor receptor-targeted kinase inhibitor, prolongs survival despite persistent brain tumor growth in mice. *J Clin Oncol* 27: 2542-2552.
107. Shaked Y, Henke E, Roodhart JML, et al. (2008) Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: implications for antiangiogenic drugs as chemosensitizing agents. *Cancer Cell* 14: 263-273.
108. Shaked Y, Tang T, Woloszynek J, et al. (2009) Contribution of granulocyte colony-stimulating factor to the acute mobilization of endothelial precursor cells by vascular disrupting agents. *Cancer Res* 69: 7524-7528.
109. Kozin S V., Kamoun WS, Huang Y, et al. (2010) Recruitment of myeloid but not endothelial precursor cells facilitates tumor regrowth after local irradiation. *Cancer Res* 70: 5679-5685.
110. Kioi M, Vogel H, Schultz G, et al. (2010) Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. *J Clin Invest* 120: 694-705.
111. Hattermann K, Held-Feindt J, Lucius R, et al. (2010) The Chemokine Receptor CXCR7 Is Highly Expressed in Human Glioma Cells and Mediates Antiapoptotic Effects. *Cancer Res* 70: 3299-3308.
112. Zagzag D, Esencay M, Mendez O, et al. (2008) Hypoxia and vascular endothelial growth factor-induced stromal cell-derived factor-1α/CXCR4 expression in glioblastomas. *Am J Pathol* 173: 545-560.
113. Batchelor TT, Duda DG, di Tomaso E, et al. (2010) Phase II study of cediranib, an oral pan-vascular endothelial growth factor receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma. *J Clin Oncol* 28: 2817-2823.

AIMS Press

6.2 ARTIGO 2

CXCL12 rs1801157 polymorphism contribution to HPV infection and cervical lesions development.

ABSTRACT

Human papillomavirus (HPV) is the most common sexually transmitted virus in women worldwide. The persistence of the virus may cause warts that are considered benign lesions, low or high grade intraepithelial lesions (LSIL/HSIL). Immunological system plays an important role in the resolution of infections. In this context, we highlight the chemokines, which are important regulators in the development of viral infections and inflammation. Among which CXCL12 stands out, due to its pro-inflammatory features, acting as chemoattractant recruiting immune cells. Several polymorphisms were identified in *CXCL12* gene including rs1801157 in the 3'-untranslated region, which is characterized by a substitution of a guanine for an adenine. In this study, 195 women were classified as HPV non-infected and 169 as HPV-infected. HPV-DNA was detected by polymerase chain reaction (PCR) and the polymorphism was assessed in blood cells through restriction fragment length polymorphism analysis. HPV infection was more incident in women who had more than 4 sexual partners during lifetime (p=0.007), among those who presented lower number of pregnancies (p=0.017). HPV was more prevalent among allele A carriers confirmed by logistic regression analysis adjusted for several confounding factors [OR_{ADJ}=4.985; $CI_{95\%}$ (2.85-8.72), p<0.001]. An association between allele A carriers and HSIL development (p=0.003) was also observed. Nonetheless, further analyses are necessary to elucidate the mechanism by which the polymorphism may be responsible for HPV infection, its influence in chemokine expression in cervical microenvironment and its role in lesion development. All in all the present study demonstrated that *CXCL12* rs1801157 is independently associated with HPV infection and exerts influence in HSIL development, suggesting it as a promising susceptibility biomarker for HPV infection and lesions development.

Keywords: CXCL12, rs1801157, HPV infection, cervical lesion

1. INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted infection in women worldwide. Infection may resist asymptomatic and is, usually, transient. Most of the women eliminate the virus from the body with the immune system effective action within 5-15 months (TROTTIER et al., 2008). The virus persistence may cause warts that are considered benign lesions, low or high grade squamous intraepithelial lesions (LSIL/HSIL) and cancer (COSER et al., 2016). Several HPV types, especially high risk types (HPV-HR), mediate squamous intraepithelial lesion (SIL) development that may progress to cervical cancer through several mechanisms such as keratinocytes malignant transformation, however many other factors contribute to the disease progression, such as tobacco use, long-duration oral contraceptive use and multiparity (GRAVITT, 2011).

Moreover immunological system plays an important role in the infection resolution. HPV-HR presence may not be elucidated and persist through several years, inducing an inflammatory microenvironment leading to pre-cancerous lesions development (ZUR HAUSEN, 2009; WOODMAN et al., 2007; AMADOR-MOLINA et al., 2013). It is known that chemokines are important regulators in the development of viral infections (MBEUNKUI; JOHANN, 2010) and are also responsible for inducing directional keratinocyte migration, notably of leukocytes during inflammation. Prolonged inflammation may facilitate carcinogenesis by providing an ideal microenvironment for tumor growth and development (VANDERCAPPELLEN et al., 2008). Several chemokines play important role in inflammation process, including CXCL12 due to its pro-inflammatory characteristic, acting as chemoattractant to immune cells such as lymphocytes (ZHOU et al., 2016).

The *CXCL12* gene is located on long arm of chromosome 10, and was first cloned from a bone marrow-derived stromal cell line and then, identified as pre-B cell growth stimulating factor (SHIROZU et al., 1995). Several polymorphisms were identified in *CXCL12* gene including rs1801157 in the 3'-untranslated region (3'UTR), described for the first time by Cheryl Winkler in 1998, and is characterized by a substitution from guanine to adenine (g.17289G>A), (WINKLER et al., 1998). This polymorphism was associated to elevated risk of some types of cancer development including breast cancer and lymphoma (DE OLIVEIRA et al., 2009).

However, to date, there is no study between *CXCL12* rs1801157 polymorphism and HPV infection as well as cervical lesions development.

In a case-control study the rs1801157 polymorphism was not associated with invasive squamous carcinoma and adenocarcinoma *in situ* (MALEY et al., 2009). On the other hand, analysis between this polymorphism genotype distribution and cervical cancer risk, showed that allele A of this polymorphism may be a risk factor for patients with a positive history of tobacco smoking (ROSZAK et al., 2015).

Due to the lack of data, we aimed to investigate the influence of *CXCL12* rs1801157 polymorphism on HPV infection and LSIL and HSIL development in a Brazilian population.

2. MATERIALS AND METHODS

2.1 ETHICAL APPROVAL AND SAMPLE CHARACTERIZATION

This study was approved by Institutional Ethics Committee Involving Humans at State University of Londrina, Londrina – Paraná (PR), Brazil (CEP/UEL 133/2012; CAAE 05505912.0.0000.5231). The study purpose and procedures were explained to all patients and written informed consent was obtained.

Between 2014 and 2016, 364 women were enrolled in this case control-study. They were recruited in health services in Londrina- PR, Brazil: the Intermunicipal Consortium of Health of the Middle Paranapanema (Cismepar), Clinical Hospital of the State University of Londrina, and from two basic health-care units. After sample collection, cytobrushes containing cervical cells were stored in 2 mL TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0) at -20°C until DNA extraction. Peripheral blood was collected with EDTA as anticoagulant, and stored at 7°C. Structured questionnaire was applied concerning sociodemographic, reprodutive and sexual behavioral data. Participants were stratified based on HPV DNA presence or absence. Cervical cytology results were collected from medical records.

2.2 GENOMIC DNA EXTRACTION

Genomic DNA was obtained from cervical cytobrushes using DNAzol (Invitrogen™ Inc., Carlsbad, CA, USA) according to the manufacturer's instructions, and from peripheral blood using Biopur Mini Spin Plus Kit (Biometrix®, Curitiba, PR, Brazil). DNA concentration was measured at 260 nm on a NanoDrop 2000c™ Spectrophotometer (Thermo Fisher Scientific, Walthan, MA, USA), and purity was assessed by sorbance ratio measured at 260 nm and 280 nm.

2.3 HPV DETECTION

HPV was detected by Polymerase Chain Reaction (PCR) using the primers MY09 (5'-CGTCCMAARGGAWACTGATC-3') and MY11 (5'-GCMCAGGGWCATAAYAATGG-3'), which are designed to amplify a conserved region of approximately 450 bp in the HPV L1 gene (BAUER et al., 1991). Reaction conditions were 190 nM of dNTPs, 500 nM of each primer, 2 mM of $MgCl_2$, 1X of Buffer (200 mM Tris-HCL, 500 mM KCl), approximately 80 ng of DNA and 1.25 U of Taq polymerase (Invitrogen™), with an annealing temperature of 55ºC. β-globin gene amplification (approximately 268 bp) was performed as an internal control, using primers GH20 (5'-GAAGAGCCAAGGACAGGTAC-3') and PC04 (5'-CAACTTCATCCACGTTCACC-3') (MARANGON et al., 2013) under the same conditions of HPV PCR. Reactions without template DNA were used as negative control to test for contamination, and DNA from HeLa cells, which are stably integrated with HPV18, was used as positive control. PCR products were electrophoresed on 10% polyacrylamide gel and stained with silver nitrate (Figure 1).

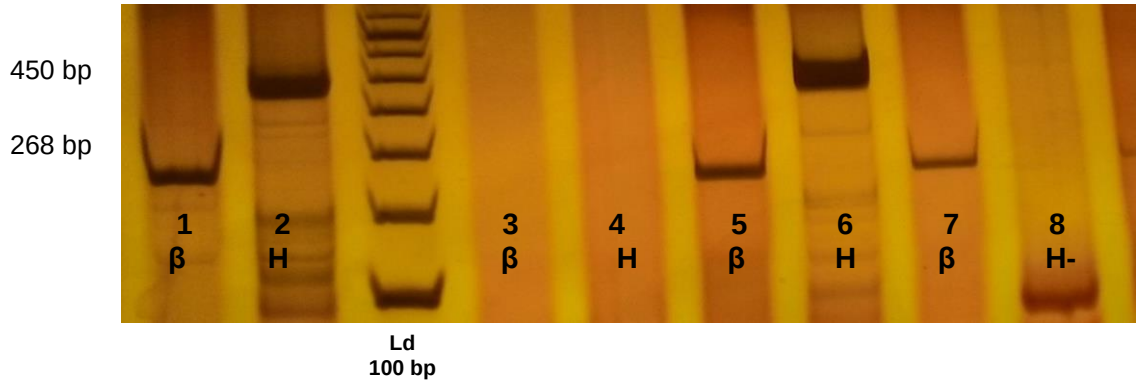

Figure 1. Electrophoretic profile of amplified HPV DNA fragment. 10% polyacrylamide gel stained with silver nitrate. β-globina, H: HPV. 1, 2- positive control; 3, 4 – negative control; 5, 6 – HPV positive patient (5:); 7, 8 – HPV negative patient

2.4 *CXCL12* rs1801157 POLYMORPHISM GENOTYPING

Genomic DNA from peripheral blood samples was used to detect *CXCL12* rs1801157 polymorphism by PCR. Primers used for *CXCL12* gene amplification were designed according to the nucleotide sequence deposited in GenBank which code is L36033. The primers forward (5' CAGTCAACCTGGGCAAAGCC 3') and reverse (5' CCTGAGAGTCCTTTTGCGGG 3') were utilized to amplify part of the 3'UTR of *CXCL12*. PCR conditions were 100 nM of dNTPs, 250 uM of each primer, 1.5 mM of $MgCl_2$, 1X of Buffer, approximately 100 ng of DNA and 1U of Taq polymerase (Invitrogen™) (Figure 2).

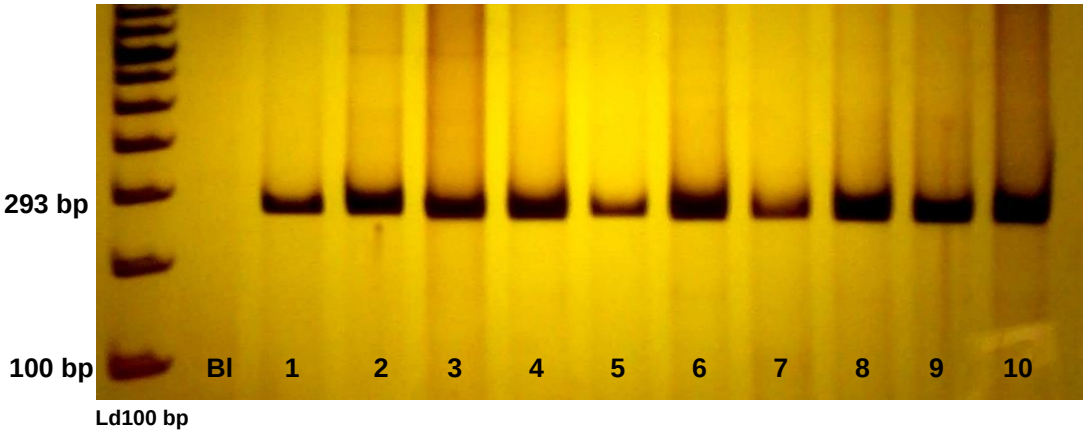

Figure 2: Electrophoretic profile of amplified CXCL12 fragment. 10% polyacrylamide gel stained with silver nitrate. L - Ladder 100 bp; Bl - negative control;1 – 10 – samples showing CXCL12 amplification.

The *CXCL12* product amplification corresponds to a 293 bp fragment. The enzymatic restriction was performed by PCR-RFLP using PCR product in the presence of the restriction enzyme *MspI* (New England Biolabs, Ipswich, MA, USA). This enzyme cleaves the amplified fragment of DNA in the presence of a guanine, producing fragments of 100 bp and 193 bp and in the presence of an adenine, the fragment of 293 bp remains intact (Figure 3).

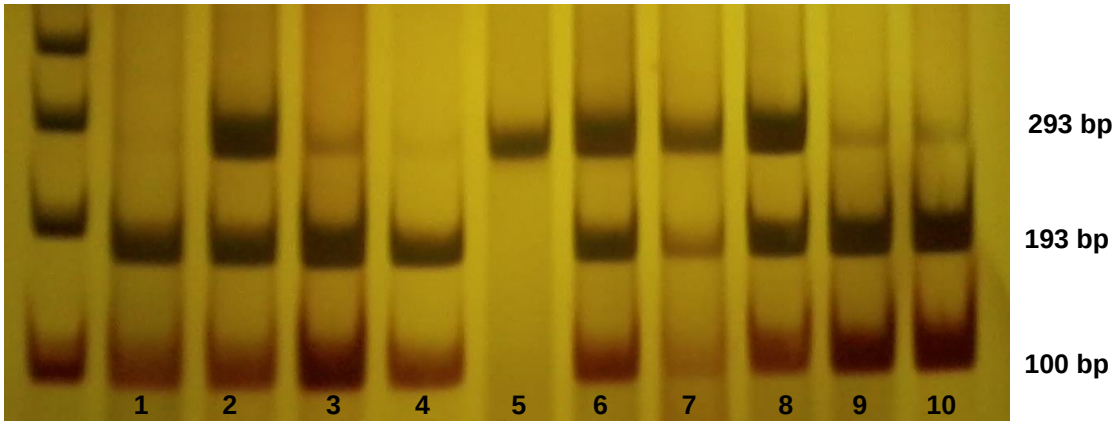

Figure 3: Electrophoretic profile of *CXCL12* rs1801157 polymorphism. 10% polyacrylamide gel stained with silver nitrate. Columns 1, 3, 4, 9, 10: GG Genotype presenting two restriction fragments, one with 100 bp and the other with 193 bp. Columns 1, 6, 7, 8: GA Genotype presenting three fragments, 100 bp, 193 pb and 293 bp. Column 5: AA Genotype showing a single fragment of 293 bp.

2.5 STATISTICAL ANALYSIS

Differences in sociodemographic and sexual behavioral data between infected and non-infected women were examined using contingency tables and Pearson's χ^2 test. Allele frequency was calculated as [1(h+2H)]/2N, where h represents the heterozygous genotype, H is the homozygous genotype, and N is the sample size for each population. Hardy-Weinberg equilibrium in infected and non-infected women was tested using χ^2 test. Differences in the distribution of genotypes were assessed by χ^2 test between non-infected and infected women, and among women with or without low- and high-grade squamous intraepithelial lesions. Adjusted Odds Ratio with 95% confidence interval was calculated to estimate the association between HPV presence, sociodemographic, reproductive and sexual behavior features, as well to analyze association of *CXCL12* polymorphism with HPV presence and lesions development. Binary logistic regression model adjusted for confounding factors was performed to establish the association between HPV presence and *CXCL12* polymorphism. All statistical analyses were performed in SPSS Statistics 22.0 (SPSS Inc., Chicago, Illinois, USA). A p value < 0.05 was considered statistically significant.

3. RESULTS

In the present study, 364 women were included and categorized as HPV non-infected patients (195/53.6%) and HPV infected patients (169/46.4%) according to the molecular detection of HPV-DNA. Non-infected women mean age was 42±12 years (median=42), while HPV infected patients mean age was 36±13 years (median=33).

Sociodemographic characteristics of both groups, HPV infected and HPV non-infected women are presented in Table 1. A higher frequency of HPV was observed in women who had no knowledge about HPV (p=0.024), were younger than 24 years old (p=0.001), single (p=0.002), smokers (p<0.001) and received less than 1 minimum wage (p=0.040).

Sexual and reproductive characteristics data are presented in Table 2. HPV infection was more incident in women who had more than 4 sexual partners during lifetime (p=0.007), among those who presented lower number of pregnancy (p=0.017).

CXCL12 rs1801157 polymorphism genotypes distribution among HPV non-infected and infected patients were in Hardy-Weinberg equilibrium (p≥0.05). A higher frequency of allele A was observed in HPV infected women (p<0.001) which was confirmed by codominant, dominant and recessive models (Table 3).

In order to confirm whether *CXCL12* rs1801157 polymorphism is associated with infection independently of confounders factors, data were adjusted for all confounding factors observed in the previous analysis in a binary logistic regression (Table 4). A significant association between allele A and HPV infection was confirmed in all the seven models proposed, indicating that the polymorphism is independently associated to HPV infection. As observed in model 7, in which data was adjusted for knowledge about HPV, age, monthly income, smoking status, number pregnancies, number of sexual partners, and marital status allele A carriers presented an increased risk for HPV infection [OR_{ADJ}=4.947; $CI_{95\%}$ (2.854-8.575), p<0.001].

Considering the polymorphism influence in lesions development, the dominant model was adopted in order to make a better distribution among genotype groups (Table 5). We observed that allele A presence was not associated to LSIL

(p=0.476) compared to women without lesion. However, it was significantly associated to HSIL (p=0.003) development.

4. DISCUSSION

To the best of our knowledge, this is the first study that demonstrated an independent association between *CXCL12* rs1801157 polymorphism HPV infection and HSIL.

According to sociodemographic data, HPV was more frequent within patients who had no knowledge about the virus which may indicated lack of information about HPV and also, the ways to avoid virus exposure; among women younger than 24 years old, single and who had more than 4 partners during lifetime.

Young age has been associated in an independent way to HPV infection (COSER et al., 2016) and it is also in accordance to Sanjosé meta-analysis (2007), probably due to the intense sexual activity among younger women, besides that, younger women usually present a larger area of ectopy compared with older adults, what means biological vulnerability to HPV infection because of the easier access to basal epithelial cells (HWANG et al., 2012).

In this study, smoking status was associated to HPV infection (p<0.001), this could be explained by the fact that tobacco smoking may cause immunosuppression (JOHNSON et al., 1990). Smoking may inhibit the immune response to HPV by decreasing Langerhans' cell in normal epithelium, moreover HPV-infected cells are exposed to tobacco carcinogens that cause DNA damage while HPV oncoprotein E6 block apoptosis (CASTLE et al., 2008). Alam et al. (2007) also reported a molecular interaction between benzo[a]pyrene (BaP), a carcinogen found in tobacco smoke, and HPV synthesis, suggesting that BaP might interfere on multiple HPV life cycle functions, such as inducing genome copies, stimulating and/or stabilizing late gene transcripts/capsid proteins and the concomitant virion assembly, potentially enhancing viral persistence, host tissue carcinogenesis, and permissiveness for cancer progression.

Lower pregnancies number (p=0.017) was also associated to HPV presence. During pregnancy, elevated estrogen and progesterone may lead to the squamous-columnar junction exposure and metaplasia. Parity might increase the risk of cervical cancer because it maintains the cervix transformation zone for many

years, facilitating exposure to HPV infection and others cofactors (MUNOZ et al., 2002). Another factor that contributes to HPV infection in pregnant women is the immunosuppression due to the steroid hormones increased levels that depress cellular immunity (BANURA et al., 2008) and may also have an effect on HPV replication. Besides, it has been shown that the transcriptional promoter of E6-E7 transforming region of HPV16 contains a steroid hormone receptor-binding element that stimulates HPV E6 and E7 transcription, suggesting a hormonal activation effect on HPV replication (GLOSS et al., 1987). Nonetheless, our data have demonstrated high risk of infection in women with no pregnancies. This may be explained by the fact that young age of our patients is correlated to lower number of pregnancies (data not shown) as well as higher risk of infection.

Genetic factors have been suggested to play a role in HPV persistence besides environmental and lifestyle factors (TAN; ANKATHIL, 2015). Virus persistence and cervical cancer risk may vary among individuals and can be partly explained by individual variations in genes involved in this complex mechanism. A combination of several genetic variants may modulate the risk factors, therefore the identification of susceptibility alleles remains a promising research field (BODILY; LAIMINIS, 2011).

In this context, we analyzed the *CXCL12* rs1801157 polymorphism in HPV infection, LSIL and HSIL development. A higher frequency of HPV was observed among allele A carriers, confirmed by binary logistic regression model adjusted for several factors as confounders, demonstrating that *CXCL12* rs1801157 is independently associated to HPV infection. Some studies observed that the polymorphism was not a risk factor for cervical cancer development (MALEY et al., 2010; ROSZAK et al., 2015; TEE et al., 2012), however, none of them have evaluated whether the polymorphism could represent a risk factor for HPV infection as demonstrated in this study.

Precursor lesions can occur as consequence of persistent infection. In the present study, allele A influence in cervical lesion development was also evaluated and a significant association was observed for allele A carriers with HSIL ($p=0.003$). Increased gradient of CXCL12 concentration was observed from LSIL to HSIL in women with HR-HPV (ZANOTTA et al., 2016).

CXCL12 has been considered as a standard proinflammatory molecule for a long time, since it attracts leukocytes to inflammatory sites contributing

to their activation (TIMOTIJEVIC, et al., 2012; ZHOU et al., 2016). Data have suggested that HPV pre-cancerous lesion depend on both the suppression of cellular immunity, driven by the Th1 response and the development of the immunosuppressive Treg profile for neoplastic progression (STRICKLER et al., 2014). Significant increased expression of CXCL12, measured by IHC and ELISA, in cervical epithelium, as the neoplastic lesion progressed from preinvasive to invasive cancer, was shown by Jaafar et al. (2009). They also showed that CXCL12 was not expressed in normal cervical squamous or glandular epithelium, which is in accordance with Zanotta et al. (2016) who have shown that healthy cervical tissue presented low or no levels of CXCL12. A particular significance correlation was found between CXCL12 and FOXP3 in cervical neoplastic lesion, suggesting that high levels of CXCL12 leads to retention or accumulation of $FOXP3^+$ T cells in progressing cervical cancer (JAAFAR et al., 2009).

Until present there are no studies about the *CXCL12* rs1801157 polymorphism influence in its expression, plasmatic or cervical levels in HPV infection or SIL development, but it has been widely studied in others diseases and tumors, showing conflicting results. De Oliveira et al. (2011) demonstrated that allele A carriers breast cancer patients have significant low levels of *CXCL12* mRNA in the peripheral blood samples when compared to GG patients. Controversially, Hirata et al. (2007) observed in prostate cancer patients that CXCL12 expression was higher in A allele than in allele G carriers.

Immunohistochemistry profile of CXCL12 in colorectal cancer showed weak or negative in normal mucosa and strongly increased in cancer tissues especially in well-differentiated tumors, 73.5% of patients that expressed a strong CXCL12 immunostaining in the membrane and cytomembrane presented AA or GA genotype. By the other hand, 88.6% of those with negative immunoreactivity presented GG genotype (AMARA et al., 2015). However, in another study with colorectal cancer patients, CXCL12 plasma levels were not related to A allele or GA / AA genotypes (DIMBERG et al., 2007).

Evidence for the involvement of the CXCL12 in the HPV life cycle arose from the abnormal and specific expression of CXCL12 observed in keratinocytes of HPV-productive skin or mucosal lesions (BALABANIAN et al., 2005).

CXCL12 expression levels may increase in keratinocytes as a consequence of HPV genome expression, generating an autocrine signaling loop

essential for keratinocyte proliferation and migration (MEURIS et al., 2016). A reasonable explanation for this mechanism is that the *CXCL12* proximal promoter in its 5'-flanking and 5'-untranslated region contain six Sp1 binding sites, and Sp1 transcription factor seems to be the major positive regulator of *CXCL12* expression (GARCÍA-MORUJA et al., 2005). Additionally, after HPV infection of basal epithelial cervical cells, E6 and E7 oncoproteins are expressed, and may bind specifically to protein 1 transcription factor (Sp1). The E6-Sp1 and E7-Sp1 complex can migrate into the nucleus and probably induce the *CXCL12* gene expression (PERALTA-ZARAGOZA et al., 2006).

Further analysis are needed in order to elucidate the polymorphism influence in chemokine expression and levels in cervix microenvironment in order to establish the immunopathological mechanism whereby CXCL12 rs11801157 polymorphism could act in HPV infection and cervical lesion development. However our work is pioneer in demonstrating the association of *CXCL12* rs1801157 polymorphism to HPV infection and HSIL development, suggesting it as a promising susceptibility biomarker for HPV infection and lesions development.

Table 1 Sociodemographic characteristics of HPV positive patients and controls.

Variable	HPV non-infected		HPV infected		*p* value*	OR	CI 95%	*p value*
	n	(%)	n	(%)				
Knowledge about HPV					**0.024**			
No	36	(19.35)	43	(32.82)		1.00	Reference	
Have ever heard	106	(57.00)	62	(47.32)		0.490	0.258-0.842	0.010
Yes	44	(23.65)	26	(19.86)		0.495	0.257-0.954	0.036
Age (years)					**0.001**			
≤ 24	14	(7.21)	30	(18.63)		1.00	Reference	
25 – 34	50	(25.77)	52	(32.29)		0.483	0.231-1.021	0.057
35 – 44	50	(25.77)	37	(23.00)		0.345	0.161-0.741	0.006
45 – 54	54	(27.85)	23	(14.28)		0.199	0.088-0.443	0.001
≥ 55	26	(13.40)	19	(11.80)		0.341	0.143-0.812	0.015
Ethnicity					0.540			
Caucasian	100	(54.06)	58	(44.61)		1.00		
Brown	61	(33.00)	60	(42.60)		1.696	1.048-2.744	0.031
Black	24	(13.00)	11	(8.50)		0.790	0.361-1.730	0.556
Asian	0	(0.00)	1	(0.80)		-	-	-
Monthly income[a]					**0.040**			
<1 minimum wage	43	(24.57)	45	(37.81)		1.00	Reference	
1 – 3 minimum wages	115	(65.71)	65	(54.62)		0.540	0.322-0.906	0.020
3 – 5 minimum wages	13	(7.42)	9	(7.57)		0.662	0.257-1.706	0.393
≥ 5 minimum wages	4	(2.27)	0	(0.00)		-	-	
Smoking status					**0.001**			
No	163	(84.90)	100	(69.90)		1.00	Reference	
Yes	29	(15.10)	43	(30.10)		2.417	1.419-4.117	0.001
Educational Stage[b]					0.488			
Until incomplete fundamental education	58	(31.40)	44	(33.90)		1.00	reference	
Complete fundamental education	17	(9.20)	18	(13.80)		1.396	0.646-3.014	0.396
Incomplete secondary education	29	(15.70)	16	(12.30)		0.727	0.352-1.502	0.390
Complete secondary education	63	(34.10)	42	(32.30)		0.879	0.505-1.528	0.647
Incomplete higher education	6	(3.20)	6	(4.60)		1.318	0.398-4.436	0.651
Complete higher education	12	(6.40)	4	(3.10)		0.439	0.133-1.455	0.178
Marital status					**0.002**			
Single	19	(9.80)	39	(24.80)		1.00	Reference	
Married / Civil partner	143	(73.70)	91	(58.00)		0.310	0.169-0.569	0.001
Divorced	23	(11.90)	19	(12.10)		0.402	0.178-0.912	0.029
Widowed	9	(4.60)	8	(5.10)		0.433	0.144-1.300	0.136

[a]Based on Brazilian minimum wage (approximately U$ 265.00). [b]Based on Brazilian educational system. *Analysis by two-sided Chi-square (X^2) test and $p<0.05$ set as significance level (SPSS Inc., Chicago, Illinois, USA). Some categories did not complete the total of patients due to lack of data.

Table 2 Sexual behavioral and reproductive characteristics of HPV positive patients and controls.

Variable	HPV non-infected		HPV infected		*p* value*	OR	CI95%	*p* value
	n	(%)	n	(%)				
Contraceptive method					0.216			
No	114	(59.70)	83	(54.20)		1.00	Reference	
Yes, hormonal	63	(33.00)	52	(34.00)		1.134	0.713-1.802	0.569
Yes, condom	13	(6.80)	13	(8.50)		1,373	0.605-3.116	0.448
Yes, both	1	(0.50)	5	(3.30)		6.867	0.788-59.882	0.081
Number of pregnancies					**0.017**			
0	15	(7.70)	26	(16.30)		1.00	Reference	
1	33	(17.00)	42	(26.30)		0.451	0.243-0.835	0.011
2	61	(31.50)	35	(21.90)		0.540	0.286-1.020	0.058
3	48	(24.50)	33	(20.60)		0.499	0.193-1.043	0.063
4	21	(10.80)	12	(7.50)		0.589	0.245-1.146	0.237
≥ 5	16	(8.50)	12	(7.40)		1.362	0.623-2.977	0.439
Abortion					0.092			
No	137	(78.28)	107	(80.45)		1.00	Reference	
Yes	38	(21.72)	26	(19.55)		0.876	0.501-1.533	0.643
Age at first sexual intercourse (years)					0.265			
≤17	102	(53.70)	89	(59.70)		1.00	Reference	
≥18	88	(46.30)	60	(40.30)		0.782	0.506-1.206	0.266
Age at menarche (years)					0.379			
≤12	89	(53,65)	82	(54,67)		1.00	Reference	
≥13	103	(46,35)	68	(45,33)		0.717	0.467-1.101	0.128
Sexual partners during the lifetime					**0.007**			
1	76	(40.60)	31	(23.80)		1.00	Reference	
2 – 3	53	(28.40)	44	(33.80)		2.035	1.142-3.628	0.016
≥4	58	(31.00)	55	(42.40)		2.325	1.332-4.059	0.003
Sexual partners within the past 6 months					0.529			
0	26	(13.90)	24	(18.20)		1.00	Reference	
1	158	(84.50)	103	(78.00)		0.706	0.385-1.297	0.262
≥2	3	(1.60)	5	(3.80)		1.806	0.389-8.382	0.451

*Analysis by two-sided Chi-square (X^2) test and $p<0.05$ as significance level (SPSS Inc., Chicago, Illinois, USA). Some categories did not complete the total of patients due to lack of data.

Table 3 Association between *CXCL12* rs1801157 polymorphism and HPV infection.

Model	HPV non-infected N (%)	HPV infected N (%)	OR	CI95%	*p* value
Codominant model					
GG	147 (75.40)	71 (42.00)	1.00		
GA	45 (23.10)	71 (42.00)	**3.25**	**2.044 – 5.22**	**<0.001**
AA	3 (1.50)	27 (16.00)	**18.63**	**5.47 – 63.49**	**<0.001**
Dominant model					
GG	147 (75.40)	71 (42.00)	1.00		
GA+AA	48 (24.60)	98 (58.00)	**4.22**	**2.70 – 6.60**	**<0.001**
Recessive model					
GG+GA	192 (98.50)	142 (84.00)	1.00		
AA	3 (1.50)	27 (16.00)	**11.80**	**3.55 – 39.56**	**<0.001**
Alleles					
G	339 (86.92)	213 (63.02)	1.00		
A	51 (13.08)	125 (36.98)	**3.90**	**2.70 – 5.63**	**<0.001**

Analysis by two-sided Chi-square (X^2) test ($p<0.05$ as significance level). OR = Odds Ratio; CI = Confidence Interval. (SPSS Inc., Chicago, Illinois, USA)

Table 4 Association study between CXCL12 rs1801157 and HPV in dominant model infection adjusted for confounder factors.

	Model 1	Model 2	Model 3	Model 4	Model
GG					
OR	1.00	1.00	1.00	1.00	1.00
CI95%	Reference	Reference	Reference	Reference	Referenc
p value					
Allele A carrier					
OR	**4.004**	**4.416**	**4.878**	**4.836**	**4.824**
CI95%	(2.472 – 6.483)	(2.677– 7.284)	(2.865– 8.308)	(2.819– 8.296)	(2.811– 8.2
p value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Logistic regression analysis with HPV as dependent variable (reference group = non-infected women) and CXCL12 rs1801157 polymorphism as explanatory variable, adjusted for several confounders according to the proposed models ($p<0.05$ as significance level). (SPSS Inc., Chicago, Illinois, USA)

Model 1: CXCL12 polymorphism adjusted for knowledge about HPV;

Model 2: CXCL12 polymorphism adjusted for knowledge about HPV and age;

Model 3: CXCL12 polymorphism adjusted for knowledge about HPV, age and monthly income;

Model 4: CXCL12 polymorphism adjusted for knowledge about HPV, age, monthly income and smoking status;

Model 5: CXCL12 polymorphism adjusted for knowledge about HPV, age, monthly income, smoking status and number of pregnancies;

Model 6: CXCL12 polymorphism adjusted for knowledge about HPV, age, monthly income, smoking status, number of pregnancies, and number of sexual partners;

Model 7: CXCL12 polymorphism adjusted for knowledge about HPV, age, monthly income, smoking status, number of pregnancies, number of sexual partners and marital status.

Table 5 Association study between CXCL12 rs1801157 allele A and lesion development

	n (%)	OR	CI95%	*p* value
LSIL				
GG	15 (57.70%)	1.000	Reference	
GA+AA	11 (42.30%)	1.347	0.594 – 3.054	**0.476**
HSIL				
GG	32 (45.10%)	1.000	Reference	
GA+AA	39 (54.90%)	2.239	1.315 – 3.811	**0.003**

Data were analyzed compared to patient without lesion. LSIL: Low-grade squamous intraepithelial lesion. HSIL: High-grade squamous intraepithelial lesion. OR: Odds Ratio. CI: Confidence Interval. (SPSS Inc., Chicago, Illinois, USA)

REFERENCE

ALAM, S. et al. Cigarette smoke carcinogen benzo[a]pyrene enhances Human Papillomavirus synthesis. **J Virol**, *82*(2), 1053–1058, 2007.

AMADOR-MOLINA, A. et al. Role of innate immunity against human papillomavirus (HPV) infections and effect of adjuvants in promoting specific immune response. **Viruses**, 5(11)2624-2642, 2013.

AMARA, S. et al. Relationship between SDF-1G801A polymorphism and its expression in tunisian patients with colorectal cancer. **Journal of Immunoassay and Immunochemistry** 36:182-194, 2015.

BALABANIAN, K. et al. WHIM syndromes with different genetic anomalies are accounted for by impaired CXCR4 desensitization to CXCL12. **Blood** *105*(6), 2449–2458, 2005.

BAUER, H. M. et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. **Jama**, *265*(4), 472–477, 1991.

BERGERS, G.; BENJAMIN, L. E. Tumorigenesis and the angiogenic switch. **Nature reviews. Cancer**, *3*(6), 401–410,2003.

BODILY, J.; LAIMINS, L. A. Persistence of human papillomavirus infection : keys to malignant progression. **Trends in Microbiology**, *19*(1), 33–39, 2011.

CASTLE, P. E. et al. How does tobacco smoke contribute to cervical carcinogenesis? **Journal of Virology**, *82*(12), 6084–6086., 2008.

COSER, J. et al. Cervical human papillomavirus infection and persistence: A clinic-based study in the countryside from South Brazil. **Brazilian Journal of Infectious Diseases**, *20*(1), 61–68, 2016.

DE OLIVEIRA, K. B. et al. CXCL12 rs1801157 polymorphism and expression in peripheral blood from breast cancer patients. **Cytokine**, *55*(2), 260–265, 2011.

DIMBERG, J. et al. Polymorphism and circulating levels of the chemokine CXCL12 in colorectal cancer patients. **International Journal of Molecular Medicine**, *19*(1), 11–15, 2007.

GARCÍA-MORUJA, C. et al. Functional characterization of SDF-1 proximal promoter. **Journal of Molecular Biology**, *348*(1), 43–62, 2005.

GRAVITT, P.E. The known unknwons of HPV natural history. **Journal of clinical Investigation**, 121(12) 4593-4599, 2011.

HIRATA, H. et al. CXCL12 G801A polymorphism is a risk factor for sporadic prostate cancer susceptibility. **Clinical Cancer Research**, *13*(17), 5056–5062, 2007.

HWANG, L. Y. et al. Cervical ectopy and the acquisition of human papillomavirus in adolescents and young women. **Obstetrics and Gynecology**, *119*(6), 1164–1170, 2012.

JAAFAR, F. et al. Correlation of CXCL12 expression and FoxP^{3+} cell infiltration with human papillomavirus infection and clinicopathological progression of cervical cancer. **Am J Pathol** 175: 1525-1535, 2009.

JOHNSON, J. D.; HOUCHENS, D. P.; WILLIAM, M. Effects of Mainstream and Environmental Tobacco Smoke on the Immune System in Animals and Humans : a Review. **Critic Rev Toxicol**, 20(5):369-95, 1990.

MALEY, S. N. et al. Genetic variation in CXCL12 and risk of cervical carcinoma: a population-based case-control study. **International Journal Immunogenet**, *36*(6), 367–375, 2009.

MARANGON, A. V. et al. The Association of the immune response genes to human papillomavirus-related cervical disease in a brazilian Population. **BioMed Research Internationa***l*, *2013*, 1–11, 2013.

MBEUNKUI F, JOHANN D J JR. Cancer and the tumor microenvironment: a review of an essential relationship. **Cancer Chemoter Pharmacol** 63: 571-582, 2010.

MEURIS, F. et al. The CXCL12/CXCR4 signaling pathway: A new susceptibility factor in human papillomavirus pathogenesis. **PLOS Pathogens,** *12*(12), 2016.

MUNOZ, N. et al. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. TT - [Rôle de la parité et du papillomavirus humain dans le cancer du col de l'utérus : étude multi-centrée de contrôle de cas de l'IARC]; **Lancet**, *359*(9312), 1093–1101, 2002.

PERALTA-ZARAGOZA, O.; BERMÚDEZ-MORALES, V. E6 and E7 oncoproteins from human papillomavirus type 16
induce activation of human transforming growth factor 1
promoter throughout Sp1 recognition sequence. **Viral Immunology**, 19(3), 468–48, 2006.

ROSZAK, A. et al. Stromal cell-derived factor-1 G801A polymorphism and the risk factors for cervical cancer. **Mol Med Rep** 4633–4638, 2015.

SANJOSÉ, S. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. **The Lancet Infectious Diseases**, v. 7, n. 7, p. 453-459, jul. 2007.

SHIROZU M. et al. Struture and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. **Genomics**: 495-500, 1995.

STRICKLER H. D. et al. The relation of plasmacytoid dendritic cells (pDCs) and regulatory T-cells (Tregs) with HPV persistence in HIV-infected and HIV- uninfected women. **Viral Immunol.** 27: 20–25, 2014.

TAN, S. C.; ANKATHIL, R. Genetic susceptibility to cervical cancer: role of common polymorphisms in apoptosis-related genes. **Tumor Biology**, *36*(9), 6633–6644, 2015.

TEE, Y. T. et al. G801A Polymorphism of Human Stromal Cell-Derived Factor 1

Gene Raises No Susceptibility to Neoplastic Lesions of Uterine Cervix. **Int J Gynecol Cancer,** *22*(8), 1297–1302, 2012.

TIMOTIJEVIĆ, G.; MOSTARICA STOJKOVIĆ, M.; MILJKOVIĆ, D. CXCL12: Role in neuroinflammation. **International Journal of Biochemistry and Cell Biology**, *44*(6), 838–841, 2012.

TROTTIER, H. et al. Type-scpecific duration of Human Papillomavirus infections: Implications for Human Papillomavirus screening and vaccination. **Journal of Infectious Disease**, 197:1436-1447, 2008.

VANDERCAPPELLEN J. et al. The role of CXC chemokines and their receptors in cancer. **Cancer Lett** 267: 226-244, 2008.

WOODMAN, C. et al. The natural history of cervical HPV infection: unresolved issues. **Nature Reviews Cancer**, 11–22, 2007.

WINKLER C. et al. Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. ALIVE Study, Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC). **Science** 279: 389-393, 1998.

ZANOTTA, N. et al. Candidate soluble immune mediators in young women with high-risk human papillomavirus infection: high expression of chemokines promoting angiogenesis and cell proliferation. **PLoS One** 11(3): e0151851, 2016.

ZHOU, W., ZHANG, J., GOLENIEWSKA, K. ET AL. Prostaglandin I2 supresses proinflammatory chemokine expression, CD4 T cell activation, and STAT6-independent allergic lung inflammation. **The Journal of Immunology,** 197(15):1577-86, 2016.

ZUR HAUSEN H. Papillomaviruses in the causation of human cancers—a brief historical account. **Virology** 384: 260-26, 2009.

7. CONCLUSÕES

ARTIGO 1:

- Embora necessária, a infecção pelo HPV não é suficiente para a imortalização e posterior malignização celular, diversos fatores podem contribuir para este processo, dentre eles as quimicionas;

- Os níveis de CXCL12 podem estar elevados em mulheres portadoras de HPV de alto risco e este nível elevado da quimiocina parece aumentar a infiltração de células T regulatórias $FOXP3^{+}$ que podem estar envolvidas na imunossupressão do microambiente do colo do útero e no desenvolvimento do câncer cervical;

- O eixo CXCL12/CXCR4 pode estar correlacionado com a metástase em linfonodos em pacientes com câncer de colo de útero através da regulação da adesão pela ativação da via de sinalização MAPK e secreção de MMP2;

- O eixo CXCL12/ CXCR4/ CXCR7 é um potencial alvo terapêutico para bloqueio das vias de sinalização que levam à metástase e à angiogênese, podendo ser combinada com a terapia adjuvante e/ou neo-adjuvante.

ARTIGO 2:

- Em relação às características sociodemográficas, mulheres com idade inferior a 24 anos, solteiras, tabagistas, e que não conhecem o HPV, mostraram-se mais susceptíveis a infecção pelo vírus. Etnia e nível de escolaridade não estiveram associados ao HPV neste estudo;

- Mulheres que tiveram múltiplos parceiros sexuais, múltiplas gestações e partos, estiveram mais expostas a infecção pelo HPV. O uso de métodos contraceptivos, aborto, idade da menarca, primeira relação sexual e número de parceiros nos últimos 6 meses não foram associados a infecção por HPV;

- O polimorfismo rs1801157 de *CXCL12* mostrou-se independentemente associado a infecção pelo vírus HPV pois mulheres portadoras do alelo A apresentaram maior infecção viral. No entanto, o polimorfismo não esteve associado ao desenvolvimento de lesões cervicais.

8. CONSIDERAÇÕES FINAIS

Embora mais estudos sejam necessários a fim de avaliar a influência do polimorfismo rs18011567 de CXCL12 no risco de infecção pelo HPV, na expressão e níveis da quimiocina CXCL12 no microambiente cervical com objetivo de elucidar quais os mecanismos imunopatológicos envolvidos na infecção e no desenvolvimento de lesões precursoras que podem levar ao desenvolvimento do câncer de colo uterino. O presente trabalho demonstrou, pela primeira vez, a associação ente o referido polimorfismo e a infecção pelo vírus HPV. Deste modo, este polimorfismo poderia contribuir como um possível marcador de susceptibilidade à infecção e ao desenvolvimento de lesões cervicais.

9. REFERÊNCIAS

ARBYN, M.; SANJOSÉ, S.; SARAIYA, M. et al. EUROGIN 2011 roadmap on prevention and treatment of HPV- related disease. **Int J Cancer**, *131*(9), 1969–1982, 2012.

APGAR, B. S.; ZOSCHNICK, L.; WRIGHT, T.C.J. The 2001 Bethesda System terminology. **American Family Physician**, v. 68, n. 10, p. 1992-1998, nov. 2003.

BAGGIOLINI, M. Chemokines in pathology and medicine. **Journal of Internal Medicine**, *250*(2), 91–104, 2001.

BALKWILL, F. Cancer and the chemokine network. **Nature Reviews Cancer**, *4*(7), 540–550, 2004.

BEAUDENON, S.; HUIBREGTSE, J. M. HPV E6 , E6AP and cervical cancer. **BMC Biochemistry**, *7*, 1–7, 2008.

BERGERS, G., BENJAMIN, L. E. Tumorigenesis and the angiogenic switch. **Nature reviews. Cance***r*, *3*(6), 401–410, 2003.

BLEUL, C. C. et al. A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). **The Journal of experimental medicine**, *184*(3), 1101–9, 1996.

BRASIL. Nomenclatura brasileira para laudos citopatológicos cervicais. **INCA**, p. 25, 2012.

BRASIL. Estimativa 2016: Incidência de Câncer no Brasil. **INCA** 2015.

BRUNI, L. et al. Cervical Human Papillomavirus Prevalence in 5 Continents: Meta-Analysis of 1 Million Women with Normal Cytological Findings. *The* **Journal of Infectious Diseases**, *202*(12), 1789–1799, 2010.

CAI, C. et al. Association of CXCL12 and CXCR4 gene polymorphisms with the susceptibility and prognosis of renal cell carcinoma. **Tissue Antigens**, *82*(3), 165–170, 2013.

CASTELLSAGUÉ, X.; MUÑOZ, N. Chapter 3: Cofactors in human papillomavirus carcinogenesis--role of parity, oral contraceptives, and tobacco smoking. **Journal of the National Cancer Institute***. Monographs*, (31), 20–8., 2003.

CHAKRAVARTI, A. To a future of genetic medicine. **Nature**, 409 (6822) 822–823, 2001.

DAVEY, D. D. et al. Bethesda 2001 implementation and reporting rates: 2003 practices of 17 participants in the College of American Pathologists Interlaboratory Comparison Program 18 in Cervicovaginal Cytology. **Archives of Pathology & Laboratory**, 128(11) 1224- 19 1229, 2004.

DE OLIVEIRA, K. B. et al. CXCL12 rs1801157 polymorphism and expression in peripheral blood from breast cancer patients. **Cytokine**, *55*(2), 260–265, 2011.

DE OLIVEIRA, K. B. et al. CXCL12 rs1801157 polymorphism in patients with breast cancer, Hodgkin's lymphoma, and non-Hodgkin's lymphoma. **Journal of clinical**

laboratory analysis, *23*(6), 387–393, 2009.

DIMAIO, D.; PETTI, L. M. The E5 proteins. **Virology**, *445*(1–2), 99–114, 2013.

DOORBAR J. Molecular biology of human papillomavirus infection and cervical cancer. ***Clin Sci*** 110: 525-541, 2006.

DOORBAR, J. Papillomavirus life cycle organization and biomarker selection. **Disease markers**, *23*(4), 297–313, 2007.

DOORBAR, J. Latent papillomavirus infections and their regulation. **CURRENT opinion in virology**, *3*(4), 416–421, 2013.

EGAWA, N. et al. (2015). Human papillomaviruses; Epithelial tropisms, and the development of neoplasia. **Viruses**, *7*(7), 3863–3890, 2015.

FAREED, M.; AFZAL, M. Single nucleotide polymorphism in genome-wide association of human population: A tool for broad spectrum service. **Egyptian Journal of Medical Human Genetics**, 14(2) 123–134, 2013.

GRIFFITH, J. W. et al. Chemokines and chemokine receptors: positioning cells for host defense and immunity. **Annual review of immunology**, *32*, 659–702, 2014.

HASSAN, S. et al. Plasma stromal cell - derived factor-1: Host derived marker predictive of distant metastasis in breast cancer. **Clinical Cancer Research,** *14*(2), 446–454, 2008.

KHADEMI, B. et al. SDF-1 and CCR5 genes polymorphism in patients with head and neck cancer. **Pathol Oncol Res**, *14*(1), 45–50, 2008.

LEE, L. J. et al. Prognostic importance of human papillomavirus (HPV) and p16 positivity in squamous cell carcinoma of the vulva treated with radiotherapy. **Gynecologic oncology**, *142*(2), 293–298, 2016.

LEVOVITZ, C. et al.TGFβ Receptor 1: An immune Susceptibility Gene in HPV-Associated Cancer. **Cancer Research**, *74*(23), 6833–6844, 2014.

MAI, S. et al. Prognostic relevance of HPV infection and p16 overexpression in squamous cell anal cancer. **International Journal of Radiation Oncology Biology Physics**, *93*(4), 819–827, 2015.

MALEY, S. N. et al. Genetic variation in CXCL12 and risk of cervical carcinoma: a population-based case-control study. **International Journal Immunogenet**, *36*(6), 367–375, 2010.

MBEUNKUI, F.; JOHANN, D. J. Cancer and the tumor microenviroment: a review of an essential relationship. **Cancer Chemoter Pharmacol** *63*(4), 571–582, 2010.

MOODY, C. A.; LAIMINS, L. A. Human papillomavirus oncoproteins: pathways to transformation. **Nature Reviews Cancer**, *10*(8), 550–560, 2010.

NGUYEN, D. X. et al. Metastasis: from dissemination to organ-specific colonization. **Nature reviews. Cancer**, *9*(4), 274–84, 2009.

PATEL, S.; CHIPLUNKAR, S. Host immune responses to cervical cancer. **Current Opinion in Obstetrics and Gynecology**, *21*(1), 54–59, 2009.

POZZOBON, T. et al. CXCR4 signaling in health and disease. **Immunology Letters**,

177, 6–15, 2016.

RAZMKHAH, M. et al. Stromal cell-derived factor-1 (SDF-1) gene and susceptibility of Iranian patients with lung cancer. **Lung Cancer** 49: 311-315, 2005.

REICHE, E. M. V. et al. The effect of stromal cell-derived factor 1 (SDF1/CXCL12) genetic polymorphism on HIV-1 disease progression. **International Journal of Molecular Medicine**, *18*(4), 785–793, 2006.

ROSZAK, A. et al. Stromal cell-derived factor-1 G801A polymorphism and the risk factors for cervical cancer. **Mol Med Rep** 4633–4638, 2015.

SCHIFFMAN, M. et al. Human papillomavirus and cervical cancer. **Lancet**, *56370*, 890–970, 2007.

SCHIFFMAN, M.; WENTZENSEN, N. Human papillomavirus infection and the multistage carcinogenesis of cervical cancer. **Cancer Epidemiology Biomarkers and Prevention**, *22*(4), 553–560, 2013.

SCHIMANSKI, C.; JORDAN, M.; SCHLAEGEL, F. et al. SNP rs1801157 significantly correlates with distant metastasis in CXCL12 expressing esophagogastric cancer. **Int J Oncol** 39: 515-920, 2011.

SEI, S. et al. Increased level of stromal cell-derived factor-1 mRNA in peripheral blood mononuclear cells from children with AIDS-related lymphoma. **Cancer Research**, *61*(13), 5028–5037, 2001.

SHIROZU, M. et al. Struture and chromosomal localization SDF1**. Genomics**, 28:495-500, 1995.

SMITH, J. S. et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: A meta-analysis update. **International Journal of Cance***r*, *121*(3), 621–632, 2007.

TEE, Y. T. et al. G801A Polymorphism of Human Stromal Cell-Derived Factor 1 Gene Raises No Susceptibility to Neoplastic Lesions of Uterine Cervix. **Int J Gynecol Cancer,** *22*(8), 1297–1302, 2012.

TORRE; L. A.; BRAY, F.; SIEGEL, R. L. et al. Global Cancer Statistics, 2012. **CA a cancer J Clin** 65: 87-108, 2015.

VANDERCAPPELLEN, J. et al. The role of CXC chemokines and their receptors in cancer. **Cancer Letters**, *267*(2), 226–244, 2008.

VIGNAL, A.; et al. A review on SNP and other types of molecular markers and their use in animal genetics. **Genetics Selection Evolution**, 34(3)275–305, 2002.

WALBOOMER, J. M. et al. Human Papillomavirus Is a Necessary Cause of Invasive Cervical Cancer. **J. pathol**, *189*(May), 12–19, 1999.

WANI, N. et al. C-X-C motif chemokine 12/C-X-C chemokine receptor type 7 signaling regulates breast cancer growth and metastasis by modulating the tumor microenvironment. **Breast Cancer Research**, *16*(3), R54, 2014.

WINKLER, C. et al. Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. **Science**, *279*(5349), 389–393, 1998.

WOODMAN, C. et al. The natural history of cervical HPV infection: unresolved issues - ProQuest. **Nature Reviews Cancer**, *7*: 11–22, 2007.

ZHOU, W.; SLINGERLAND J. M. Links between estrogen receptor activation and proteolysis relevance to hormone-regulated cancer therapy. **Nat Cancer Review**, 14:26-38

ZLOTNIK, A.; YOSHIE, O. The Chemokine Superfamily Revisited. **Immunity**, *36*(5), 705–712, 2012.

ZUR HAUSEN, H. Papillomaviruses in the causation of human cancers - a brief historical account. **Virology**, *384*(2), 260–265, 2009.

APÊNDICE A

Termo de Consentimento Livre e Esclarecido

"Prevalência e genotipagem de HPV e sua possível associação com os genes de citocinas, quimiocinas e seus receptores em nível de DNA, RNA e proteína: implicações no microambiente tumoral."

Prezado(a) Senhor(a):

Gostaríamos de convidá-lo (a) a participar da pesquisa **"Prevalência e genotipagem de HPV e sua possível associação com os genes de citocinas, quimiocinas e seus receptores em nível de DNA, RNA e proteína: implicações no microambiente tumoral."**, realizada no **"Laboratório de Genética Molecular e Imunologia, Departamento de Ciências Patológicas da Universidade Estadual de Londrina".** O objetivo da pesquisa é avaliar a presença do vírus em mulheres atendidas em programas de prevenção ao câncer cervical do setor público de saúde da região norte do Paraná, por meio de metodologia específica e sensível, visando também à associação de dados demográficos, para análise dos fatores de risco que contribuem para a exposição da população ao vírus, bem como os determinantes de sua manutenção. Adicionalmente objetiva-se compreender o papel do sistema imune no controle e iniciação tumoral, bem como na sua formação, crescimento e progressão, em especial avaliar a interação tumor-hospedeiro em pacientes portadoras do vírus HPV e no desenvolvimento do câncer cervical**.** A sua participação é muito importante e ela se daria da seguinte forma: **doação de 5mL de sangue periférico coletado por punção venosa e doação do swab cérvico-vaginal utilizado para confecção das lâminas para o exame preventivo para anállises moleculares, bem como responder um questionário sociodemográfico.** Gostaríamos de esclarecer que sua participação é totalmente voluntária, podendo você: recusar-se a participar, ou mesmo desistir a qualquer momento sem que isto acarrete qualquer ônus ou prejuízo à sua pessoa. Informamos ainda que as informações serão utilizadas somente para os fins desta pesquisa e serão tratadas com o mais absoluto sigilo e confidencialidade, de modo a preservar a sua identidade.
As amostras biológicas (sangue periférico e secreção cérvico-vaginal) serão utilizados para extração de DNA e RNA para análises moleculares e imunológicas. Estes materiais serão obtidos em pequenas quantidades portanto não haverá sobra de material biológico.
Os benefícios esperados são a detecção precoce do vírus HPV em mulheres atendidas em programas de prevenção ao câncer de colo de útero do setor público de saúde da região norte do Paraná. Informamos que a paciente que se dispor a participar do projeto não sofrerá desconfortos nem riscos à saúde, não havendo qualquer prejuízo às mesmas. Informamos que a senhora não pagará nem será remunerada por sua participação. Garantimos, no entanto, que todas as despesas decorrentes da pesquisa serão ressarcidas, quando devidas e decorrentes especificamente de sua participação na pesquisa.
Caso você tenha dúvidas ou necessite de maiores esclarecimentos pode nos contactar **Karen Brajão de Oliveira, Laboratório de Genética Molecular e Imunologia, Departamento de Ciências Patológicas, Universidade Estadual de Londrina, 3371-4267, karen.brajao@uel.br),** ou procurar o Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, na Avenida Robert Kock, nº 60, ou no telefone 33712490. Este termo deverá ser preenchido em duas vias de igual teor, sendo uma delas, devidamente preenchida e assinada entregue a você.

Londrina, ___ de _____de 201__.

Pesquisador Responsável _____
Profª. Drª. Karen Brajão de Oliveira
RG:: 6.538.742-5

_____ (nome por extenso do sujeito de pesquisa), tendo sido devidamente esclarecido sobre os procedimentos da pesquisa, concordo em participar **voluntariamente** da pesquisa descrita acima.

Assinatura (ou impressão dactiloscópica):_____
Data:_____

13. Idade da 1ª relação sexual: _____anos

DICE B

ANEXO A

AUTORIZAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS/UEL

Universidade Estadual de Londrina

PARANÁ
GOVERNO DO ESTADO

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS
Universidade Estadual de Londrina
Registro CONEP 5231

Parecer CEP/UEL:	133/2012
CAAE:	05505912.0.0000.5231
Processo:	19275/2012
Pesquisador(a):	Karen Brajão de Oliveira
Unidade/Órgão:	CCB – Departamento de Ciências Patológicas

Prezado(a) Senhor(a):

O "**Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina**" (Registro CONEP 5231) – de acordo com as orientações da Resolução 196/96 do Conselho Nacional de Saúde/MS e Resoluções Complementares, avaliou o projeto:

"PREVALÊNCIA E GENOTIPAGEM DE HPV E SUA POSSÍVEL ASSOCIAÇÃO COM OS GENES DE CITOCINAS, QUIMIOCINAS E SEUS RECEPTORES EM NÍVEL DE DNA, RNA E PROTEÍNA: implicações no microambiente tumoral."

Situação do Projeto: **Aprovado**

Informamos que deverá ser comunicada, por escrito, qualquer modificação que ocorra no desenvolvimento da pesquisa, bem como deverá ser encaminhado ao CEP/UEL relatório final da pesquisa, conforme prevê a Resolução 196/96 do Conselho Nacional de Saúde/MS e Resoluções Complementares.

Londrina, 28 de agosto de 2012.

Profa. Dra. Alexandrina Aparecida Maciel Cardelli
Coordenadora do Comitê de Ética em Pesquisa Envolvendo Seres Humanos
Universidade Estadual de Londrina

Profª Drª Paula Mariza Zedu Alliprandini
Vice-Coord. do Comitê de Ética em Pesquisa
Envolvendo Seres Humanos
Universidade estadual de Londrina