LUCIANA OLIVEIRA DA SILVA

**EXPRESSÃO DO FATOR DE CRESCIMENTO ENDOTELIAL
VASCULAR (VEGF) E SEUS RECEPTORES VEGFR-1 E VEGFR-2
DURANTE O INÍCIO DA GESTAÇÃO EM CAMUNDONGOS**

**Dissertação apresentada ao Programa de
Pós-Graduação em Biologia Celular e
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da Universidade de São Paulo, para obtenção
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Área de concentração:

Biologia Celular e Tecidual

Orientador:

Prof. Dr. Sérgio Ferreira de Oliveira

**São Paulo
2008**

RESUMO

Silva LO. Expressão do Fator de Crescimento Endotelial Vascular (VEGFA) e seus receptores VEGFR-1 e VEGFR-2 durante o início da gestação em camundongos [tese]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2008.

Em roedores, o aumento da permeabilidade vascular, a transformação decidual, e angiogênese são eventos cruciais para o sucesso da gestação. O fator endotelial vascular (VEGF) é um mitogênico para células endoteliais e um indutor de angiogênese. O VEGF age via dois receptores da família das tirosinas quinases: VEGFR-1(Flt-1) e VEGFR-2 (KDR/Flk-1). Embora as células endoteliais sejam o principal alvo do VEGF, em células não endoteliais, o VEGF e seus receptores têm demonstrado exercer um efeito funcional sobre a proliferação e diferenciação celular. O objetivo deste estudo foi investigar usando o método imunohistoquímico, a expressão espacial e temporal do VEGF e seus receptores VEGFR-1 e VEGFR-2, em células endometriais de camundongo entre o 4º e 8º dias de gestação. No 4º dia de gestação, VEGF, VEGFR-1 e VEGFR-2 foram expressos pelo epitélio luminal e glandular. Células do estroma endometrial e o miométrio mostram uma marcação muito fraca. Do 5º ao 8º dias de gestação, o VEGFR-1 foi expresso pelas células do epitélio luminal e glandular e mostrou um aumento e uma marcação uniforme por toda a decídua mesometrial e antimesometrial. O receptor VEGFR-1 foi altamente expresso pelas células endoteliais dos capilares sinusóides mesometriais. A expressão de VEGFR-2 nos tecidos uterinos mostrou o mesmo padrão de marcação que aquele do VEGFR-1, exceto pela diminuição de marcação nas células da decídua madura antimesometriais. O receptor VEGFR-2 mostrou uma intensa marcação nas células pré-decíduais mesometriais e antimesometriais. O receptor VEGFR-2 foi altamente expresso pelas células endoteliais dos capilares sinusóides mesometriais. Em contraste com o endométrio decidualizado, a marcação para VEGF, VEGFR-1 e VEGFR-2 foi negativa no estroma endometrial dos sítios de inter-implantação. No 7º e 8º dias de gestação, a marcação para o VEGF, VEGFR-1 e VEGFR-2 e os seus receptores foi intensa nas células Nk uterinas. VEGF, VEGFR-1 e VEGFR-2 foram diferencialmente expressos pelas células trofoblásticas gigantes e pelas células do cone ectoplacentário sugerindo que o VEGF possa estar envolvido na invasão e diferenciação do trofoblasto. Estes resultados confirmam observações prévias que indicam

que o aumento da expressão de VEGF, VEGFR-1 e VEGFR-2 pelas células decíduais e células em processo de decidualização possam estar relacionado a um aumento de permeabilidade que ocorre durante a implantação e decidualização. Os resultados também sugerem que durante a decidualização, além das funções angiogênicas, O VEGF e os seus receptores possam está envolvidos em outros processos celulares tais como, proliferação, sobrevivência e/ou diferenciação da célula decidual.

Palavras Chaves: VEGF; VEGFR-1; VEGFR-2; Imunohistoquímica; Endométrio; Decídua; Angiogênese; Implantação embrionária.

ABSTRACT

Silva LO. Expression of vascular endothelial growth factor (VEGF) and its receptors VEGFR-1 and VEGFR-2 during early pregnancy in mice [thesis]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2008.

In rodents, increase of vascular permeability, decidual cell transformation, and uterine angiogenesis are crucial events for the success of pregnancy. Vascular endothelial growth factor (VEGF) is a mitogen for endothelial cells and an inducer of angiogenesis. VEGF acts via two tyrosine kinase family receptors: VEGFR-1(Flt-1) and VEGFR-2(KDR/Flk-1). Although endothelial cells are the main target of VEGF, in some nonendothelial cells VEGF and its receptors have shown functional effects on cell proliferation and differentiation. The aim of this study was to investigate using the immunohistochemical method, the spatiotemporal expression of VEGF and its receptors VEGFR-1 e VEGFR-2 by mouse endometrial cells on days 4 to 8 of pregnancy. On day 4, VEGF, VEGFR-1 and VEGFR-2 were expressed mostly by the luminal and glandular epithelium. Stromal cells and the myometrium showed a very weak labeling. On days 5-8, VEGF and its receptors showed an increased and uniform labeling throughout the mesometrial and antimesometrial decidua. The expression of VEGF, VEGFR-1, and VEGFR-2 was differentially expressed in the mesometrial cells and in the predecidual cells of the antimesometrial decidua. On days 5-8, VEGFR-1 was expressed by the luminal and glandular epithelium, and showed an increased and uniform labeling throughout the antimesometrial and mesometrial decidua. The receptor VEGFR-1 was highly expressed by endothelial cells of the mesometrial sinusoids. The expression of VEGFR-2 in the uterine tissues followed the same pattern of that from VEGF-R1, excepting by the decrease of labeling on antimesometrial mature decidual cells. The receptor VEGFR-2 showed a highly positive staining in the antimesometrial and mesometrial predecidual cells. The receptor VEGFR-2 was also highly expressed by endothelial cells of the mesometrial sinusoids. In contrast, endometrial stromal cells from interimplantation sites were negative for VEGF, VEGFR-1, and VEGFR-2. The expression of VEGF, VEGFR-1, and VEGFR-2 were intense in the uterine NK cells during days 7 and 8 of pregnancy. VEGF, VEGFR-1, and VEGFR-2 was differentially expressed by trophoblast giant cells and cells of the ectoplacental cone

suggesting that VEGF may be involved in trophoblast invasion and differentiation. These results confirm previous observations indicating that the increased expression of VEGF, VEGFR-1, and VEGFR-2 by decidual cells and cells in processs of decidualization may be related to an increase of vascular permeability that occurs during implantation and decidualization. The results also suggest that during decidualization, besides their angiogenic functions, VEGF and its receptors may be involved in other cell processes such as decidual cell differentiation, proliferation and/or cell survival.

Key Words: VEGF; VEGFR-1; VEGFR-2; Immunohistochemistry; Endometrium; Decidua; Angiogenesis; Embryo implantation.

INTRODUÇÃO

A implantação embrionária em mamíferos consiste de uma seqüência de eventos que se inicia com a adesão do blastocisto ao epitélio uterino. Este processo é acompanhado de uma série de modificações morfológicas, hormonais e bioquímicas que ocorre entre dois organismos geneticamente diferentes (Weitlauf, 1994).

Em roedores, um dos primeiros sinais da implantação embrionária é caracterizado por um edema generalizado, e pelo aumento da permeabilidade vascular dos vasos sanguíneos situados no estroma uterino subjacente à câmara de implantação onde se aloja o blastocisto (Psychoyos, 1961).

Outro evento que acompanha a implantação embrionária em roedores é a extensa remodelação dos tecidos maternos, conhecido como reação decidual ou decidualização (Abrahamsohn, et al., 2002).

Durante a decidualização em roedores, o endométrio forma dois compartimentos morfológicamente distintos: o tecido decidual anti-mesometrial, ou decídua antimesometrial, local onde se inicia o processo de implantação do embrião; e o tecido decidual mesometrial, ou decídua mesometrial, local de intensa angiogênese, e onde o trofoblasto invade. Nesta região, posteriormente se formará a porção materna da placenta, permanecendo até o final da gestação (Welsh and Enders, 1991b; Gu, et al., 1992; Christofferson, et al., 1994, Tan, et al., 1999).

Dentre as diversas respostas uterinas à implantação do blastocisto e á decidualização do endométrio destaca-se o aumento da permeabilidade vascular (Finn, 1977; Parr & Parr, 1989; Abrahamsohn & Zorn, 1993). Tanto o aumento da permeabilidade vascular quanto a angiogênese são eventos cruciais para o sucesso da implantação embrionária e da placentação (Ma, et al., 2001). No entanto, os mecanismos moleculares pelos quais o aumento da permeabilidade vascular e a angiogênese que ocorrem no útero ao longo das diferentes etapas da reprodução e durante o processo de decidualização são ainda pouco compreendidos.

O fator de crescimento endotelial (VEGF) é um potente mitogênico para as células endoteliais e um potente indutor de angiogênese para células endoteliais derivadas de artérias, veias e vasos linfáticos (Ferrara e Davis-Smith, 1997 Dvorak, et al., 1999). O VEGF, também é referido como VEGF-A ou VPF (Fator de Permeabilidade Vascular), pertence a uma família de genes que incluem também o fator de crescimento placentário (PlGF) (Nagy, et al., 2003), VEGF-B (Olofsson, et al., 1996), VEGF-C e o VEGF-D. Estes dois últimos representantes, C e D estão associados à regulação do processo de angiogênese nos vasos linfáticos (Nagy, et al., 2003).

O VEGF exerce sua função via dois receptores da família da tirosina quinase, o c-fms-like tirosina kinase (Flt-1: VEGFR-1) (Shibuya, et al., 1990) e o fetal liver kinase (Flk-1 ou KDR em humanos: VEGFR-2) (Millauer, et al., 1993), os quais são expressos pelas células endoteliais. Recentes estudos imunohistoquímicos têm mostrado que além das células endoteliais, os receptores Flk-1 e Flt-1 estão também localizados no epitélio glandular, e no estroma endometrial humano (Meduri, et al., 2000). O VEGF também interage com uma família de co-receptores, as neuroporinas, NP1 e NP2 aumentando a afinidades dos receptores VEGFR-2 e VEGFR-1 a isoforma do VEGF₁₆₅ (Soker et al., 1998; Ferrara, 2004).

A expressão de VEGF e seus receptores tem sido demonstrada durante o ciclo sexual em humanos e em ratos, e nos estágios iniciais da gestação em camundongos (Charnock-Jones, et al., 1993; Shweiki, et al., 1993; Chakraborty, et al., 1995; Wang, et al., 2003). Estudos realizados durante a gestação em ratas mostrou que a expressão de VEGF é regulada pelos níveis de estrógeno e progesterona peri-implantacionais (Shweiki, et al., 1993).

Apesar de vários estudos sobre VEGF e seu papel na formação de novos vasos sanguíneos, e no aumento da permeabilidade vascular terem sido publicados, pouco ainda se sabe onde este fator é expresso exatamente no útero gravídico, especialmente durante o processo de decidualização em camundongos.

CONCLUSÃO

No presente estudo ficou demonstrado que o endométrio de camundongos expressam o VEGF e os receptores VEGF-R1 e VEGF-R2 durante o processo de transformação decidual. Esta conclusão principal foi baseada nas seguintes observações:

1. O VEGF foi expresso de maneira espacial e temporal nas células do estroma uterino localizado em torno do embrião, nas células do epitélio luminal e glandular entre o 4º e o 8º dias de gestação.
2. A expressão de VEGF, VEGF-R1 e VEGF-R2 no endométrio no 4º. dia de gestação, período em que antecede a implantação do blastocisto, foi mais expressiva no epitélio luminal e glandular do que nas células do estroma sub-epitelial.
3. No 5o. dia de gestação, em resposta à implantação do blastocisto na parede uterina, ocorreu um aumento da expressão de VEGF, VEGF-R1 e VEGF-R2 tanto no epitélio luminal e glandular quanto no estroma mesometrial e na recém estabelecida decídua antimesometrial.
4. A análise imunohistoquímica mostrou que células deciduais maduras e células pré-deciduais mesometriais expressam intensamente o VEGF entre o 6º e o 8º dias de gestação. Na decídua antimesometrial, a expressão do VEGF foi semelhante aquela da decídua mesometrial, exceto pela diminuição da marcação nas células da decídua madura.
5. Do 5º ao 8º. dias de gestação, o receptor VEGF-R1 mostrou uma marcação uniforme por toda a decídua mesometrial e antimesometrial. O receptor VEGF-R1 foi altamente expresso pelas células endoteliais dos capilares sinusóides mesometriais.
6. A expressão de VEGF-R2 no endométrio decidualizado mostrou o mesmo padrão de marcação semelhante ao do VEGF-R1, exceto pela diminuição de marcação nas células da decídua madura. Assim como o receptor VEGF-R1, o receptor VEGF-R2 foi intensamente expresso pelas células endoteliais dos capilares sinusóides mesometriais.

7. Em contraste com a marcação no endométrio decidualizado, a marcação para VEGF, VEGF-R1 e VEGF-R2 foi negativa no estroma endometrial dos sítios de inter-implantação no 7º e 8º. de gestação.

8. No 7º e 8º. dias de gestação, a marcação para o VEGF, VEGF-R1 e VEGF-R1 foi intensa nas células Nk uterinas.

9. VEGF, VEGF-R1 e VEGF-R2 foram diferencialmente expressos pelas células trofoblásticas gigantes e pelas células do cone ectoplacentário.

Em resumo, a expressão diferencial do VEGF e dos receptores VEGF-R1 e VEGF-R2 na decídua mesometrial e antimesometrial e a ausência de marcação no estroma não decidualizado, nos sítios de inter-implantação demonstrou claramente que o VEGF, VEGF-R1 e VEGF-R2 são supra-regulados durante o processo de transformação decidual.

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