**Multifunctional angiogenic factors: add GnRH to the list. Focus on “Gonadotropin-releasing hormone-regulated chemokine expression in human placentation”**

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The historical view that angiogenesis is governed by the action of a few dominant trophic factors, such as VEGF, FGF, and transforming growth factor-β (TGF-β), has steadily eroded over the past two decades to give rise to the vision of a much more dynamic regulation in which angiogenesis is balanced among multiple angiogenic and angiostatic influences (5, 19). Equally important has been the identification of multiple varieties of molecules that influence this balance but are not primarily angiogenic factors themselves and have other critical regulatory functions. Examples of this latter class include integrins (21), nerve growth factor (17), brain-derived neurotrophic factor (9), hepatocyte growth factor (25), and many others. Not surprisingly, angiogenesis is also subject to modulation by interstitial products of inflammation, such as cytokines of both the interleukin (16) and CXC (22) families. Several types of lymphocytes also influence angiogenesis (3, 7, 22). In light of this complexity, it is important to ask how the multiple facets of angiogenesis are coordinated and what molecules are in charge? The recent study by Babwah and colleagues (1) addresses these questions, and for the angiogenic processes involved in uterine spiral artery remodeling during human placentation, the authors’ evidence reveals gonadotropin-releasing hormone (GnRH) to be a multifunctional coordinating player.

Development of the human placenta begins near the end of the first trimester with the invasion of cytotrophoblasts into the uterine wall to anchor the embryonic chorion to the endometrium (12). Differentiation of the invading cytotrophoblasts yields extravillous trophoblasts (EVTs), which initiate remodeling of the uterine spiral arteries by promoting the programmed cell death of smooth muscle and endothelial cells (11). Whereas this basic sequence has been known for many years (14), its molecular regulation remains unclear. The involvement of GnRH in human placentation was recognized more than a decade ago, but its role was originally associated with regulation of matrix metalloproteinase activity (18). Similarly, involvement of lymphoid cells in placentalization was proposed more than two decades ago (20), but the idea that a major subset of these cells, the uterine natural killer cells, could produce and release pro-angiogenic factors, is a much more recent idea (10) and one with uncertain physiological significance. A role for chemokines in placentalization was also proposed several years ago (15), as was the idea that CXC chemokines might play a role in angiogenesis (13) but direct evidence of a role for CXC chemokines during placentalization has been lacking.

Reasoning that the main effects of GnRH on placentalization could be detected at the mRNA level in EVTs, Babwah and colleagues treated an immortalized human placental trophoblast cell line (human HTR-8/SVneo trophoblasts) with buserelin (a synthetic GnRH-I analogue), GnRH-II, or antide (a GnRH-I competitive antagonist) for varying intervals at physiologically relevant concentrations, and then they screened for changes in gene expression using an Affymetrix microarray. This screen identified 37 genes of which the expression changed at least 1.5-fold in response to GnRH manipulation. Among these genes were the pro-angiogenic chemokines CXCL2, CXCL3, CXCL6, and CXCL8. To verify that these chemokines were responsive to GnRH, the HTR-8/SVneo cell line experiment was repeated with mRNA analysis performed via qPCR. In addition to verifying the microarray results, the qPCR measurements also established a timecourse for gene expression that was what has been observed for GnRH-promoted matrix metalloproteinase responses (2). To be sure that these results were not a unique feature of the HTR-8/SVneo cell line, the authors used immunofluorescence to identify expression of CXCL8 within trophoblasts in sections of fixed human placenta. Other experiments revealed that GnRH treatment of cultured trophoblasts enhanced the release of CXCL8 into the culture media. Together the results clearly implicate GnRH as a regulator of angiogenic chemokines in placental trophoblasts.

Given the connection between GnRH stimulation and CXC chemokine release from trophoblasts, Babwah and colleagues next explored the hypothesis that the chemokine release stimulated by GnRH helps to mediate lymphocyte recruitment during placentation. To test this possibility, the migration of fluorescently labeled Jurkat T cells, purified CD4+/CD8+ T cells, and uterine natural killer cells was measured using transwell assays of chemotaxis. Conditioned media from the HTR-8/SVneo cell line and primary EVTs induced similar and potent migration responses in each of the lymphocyte populations tested. Most importantly, the GnRH receptor antagonist antide blocked the effect of GnRH on lymphocyte recruitment, thus confirming the critical role of this receptor as a mediator of GnRH actions in the placenta. In parallel, the effect of GnRH on lymphocyte recruitment was also blocked by repertaxin, an allosteric noncompetitive inhibitor of the CXCR1 and CXCR2 receptors that mediate the intracellular effects of CXCL2, CXCL3, CXCL6, and CXCL8. Interestingly, however, the ability of repertaxin to inhibit recruitment was more pronounced for uterine natural killer cells than for the Jurkat T cells, suggesting an important difference in how migration is regulated in the two cell types. Another important feature of these results was the continued slow recruitment of lymphocytes even in the presence of repertaxin. This residual recruitment suggests that the CXCR1 and CXCR2 receptors do not mediate all lymphocyte recruitment and perhaps that other chemokines (e.g., CXCL12 or CXCL16) and receptors (e.g., CXCR4 or CXCR6) are also involved (8, 24). Even so, the results from Babwah and colleagues provide convincing new
support for the hypothesis that GnRH produced during trophoblast invasion of the endometrium stimulates the synthesis and release of multiple pro-angiogenic chemokines, which in turn serve to recruit lymphocytes that reinforce the spiral artery remodeling process essential for successful implantation.

As for many important advances, more questions are raised than answered by the Babwah study. In particular, which other chemokines help recruit lymphocytes to the site of implantation; are any of these also influenced by GnRH? How do interleukins and other members of the gp130 or TGF-β families interact with the CXC chemokines during placentaion (4); do these interactions alter either recruitment of lymphocytes or placental development? How are the CC chemokines integrated into this overall process (6)? Might physiological concentrations of GnRH also alter local expression of vascular endothelial growth factor, as has been observed clinically in response to pharmacological doses of GnRH (23)? What other functions are served by the 37 trophoblast genes of which the expression changes in response to GnRH, and what impact might GnRH have on the genomes of non-trophoblast cells of the uterus and conceptus? Given that many lymphocytes can exert a broad variety of angiogenic influences depending on their state of activation (3), it will be important to know whether the implantation environment somehow programs recruited lymphocytes to express an optimal angiogenic profile, and if so, does GnRH help control this process? Because the GnRH receptor activates different signal transduction pathways in pituitary and extrapituitary tissues (1a), it will also be important to learn whether the coupling of GnRH receptor activation to cytosolic kinases, phosphatases, and transcription factor activation are modulated or specialized in certain cell types during trophoblast invasion and placental development. More simply, better understanding of the molecular and genetic events that lead to increased GnRH expression will enlighten the search for the global factors that govern the overall implantation process.

Aside from the remaining questions regarding the role of GnRH in cytotrophoblast invasion and placental development, it is clear that GnRH is an important regulatory molecule with multiple cell-type specific functions. Because these functions include a major effect on angiogenesis during implantation, GnRH is an excellent example of a multifunctional angiogenic factor. As such, it represents a growing class of molecules that exert simultaneous complementary effects on parenchymal and vascular elements in a given tissue. In turn, the study by Babwah and colleagues is a strong example of how to identify such regulatory molecules and elucidate the mechanisms they govern.

REFERENCES


