Correspondence

The Role of Steroid Hormones on the Regulation of Vascular Endothelial Growth Factor

To the Editor-in-Chief:

I read with interest the recent correspondence between Soares et al and Iruela-Arispa and Graubert¹ on regulation of vascular endothelial growth factor (VEGF) by steroid hormones in uterine and breast cancer cells. Since VEGF is a very potent angiogenic growth factor and a potential molecular target for steroid receptor-based anti-hormone therapy, it is important to understand the role of steroid hormone receptors in the regulation of this gene. However, current studies in the literature remain controversial with regard to the role of steroid hormones on regulation of the VEGF gene. Our group has analyzed VEGF expression by steroid hormones in breast and uterine cells over the past 10 years, and we have published some results that differ with those presented by the authors of the letters.¹ I would like to add a few comments to those already proposed in the letters, and propose some reasons for discrepancies in the literature related to the role of steroid hormones and VEGF regulation. In addition, I would like to briefly comment on the role of progesterone, and synthetic progestins in the regulation of VEGF, especially since recent evidence links the use of estrogen/progestin combination hormone replacement therapy with higher incidence of breast cancer than the use of estrogen alone.^{2,3} The role of progesterone in regulating angiogenic growth factors is just beginning to emerge, and may have a role in the progression of tumor following hormone administration.

Soares et al describe in their unpublished results that VEGF mRNA is "overexpressed" by estradiol in MCF-7 breast cancer cells.¹ Similar to results of Soares et al,¹ a 4- to 6-fold induction of VEGF by estrogen is reported in the MCF-7 cells by other investigators.⁴ Furthermore, unlike the "overexpressed" or modest induction of VEGF by estrogens reported in breast cancer cells,^{1,4} we and others⁵⁻⁷ and our unpublished observations have not been able to demonstrate estrogen induction of VEGF in estrogen receptor (ER)-positive MCF-7, T47-D, or ZR-75 breast cancer cells at either mRNA level (measured at 3 to 6 and 24 hours after estrogen exposure), or protein level (enzyme-linked immunosorbent assay after 18 hours, to measure secretory forms of VEGF in media). Most genes under direct regulation by estradiol are up-regulated in this time frame even *in vivo*, including VEGF in the uterus.⁸ Thus, although we and others have described estrogen response elements in the regulatory regions of the VEGF gene,^{9,10} the data in the literature clearly indicates that estrogen induction of VEGF is cell

type-dependent and factors other than ER are also involved in the estrogen response. Fluctuation of such factors in cell lines in different laboratories could explain some discrepancies between the results of various investigators using similar experimental conditions. Identification of such additional factors may be important to fully understand the role of estrogen in VEGF response, and may even provide new prognostic factors for endocrine responsive tumors.

In one respect, the results of Soares et al¹ may be very important, and potentially may be describing an indirect effect of estrogen on VEGF regulation that they observe after 48 hours. Therefore, it is important that more details about their experimental conditions be made available to understand the importance of such an observation. At present, without evidence for use of anti-hormones to block VEGF induction, the best we can do is to conclude (as Soares et al have done) that the results of Soares et al with MCF-7 cells describe an indirect effect of E on VEGF production.

With respect to the effects of estrogen on VEGF expression in the uterus, our results, and those of others, differ considerably from the results reported by Iruela-Arispa in human uterus.¹ While Iruela-Arispa describe a modest induction (1.6-fold) of this growth factor in human uterine cells in response to estrogen,¹ we and others have seen substantial induction of mRNA for this growth factor in the rat uterus.^{11,12} Unlike the conclusions reached by Iruela-Arispa on the role of ER in VEGF induction, there is considerable evidence that, in rat uterine cells, ER is one of the components controlling estrogen-induced VEGF production.^{11,13} It is surprising that, while all uterine cell types contain ER, only the stromal cells respond to estrogen for VEGF induction.^{11,14} This suggests that, like the cell-specific effects of estrogen on VEGF regulation in the breast, additional factors are also required for ER-dependent induction of VEGF in the various cell types of the uterus. To complicate the matters even more, some investigators have found negative regulation of VEGF by estrogen in uterine and breast tumor cell lines.^{7,15} It must be stressed that while the conflicting results of VEGF regulation by estrogens is mostly derived *in vitro* from tumor cells, where the expression of co-factors may vary tremendously, the evidence from normal uterine cells is derived from intact uterus where fluctuation in any co-factors may have been minimized.^{11,13} The presence of such co-factors may also be species-dependent leading to some of the variability reported with respect to sex steroid control of VEGF expression. We cannot rule out that there may even be different degrees of mRNA stabilization in various tissues leading to much higher levels of VEGF message being observed in response to estrogen administration in certain cases.

Iruela-Arispa suggest that VEGF may not be regulated by estrogens in the human uterus because the estrogen levels are low during the first 5 days following menstruation.¹ How-

ever, we have determined that in the rat uterus, unlike many other estrogen-responsive genes, VEGF is exquisitely sensitive to low levels of estrogen and potentially could be up-regulated at very low concentrations.⁸ While admittedly speculative, expression of VEGF may be supersensitive to estrogens and respond to low levels of hormones immediately following menstruation. This remains to be proven but should be considered for future studies.

An important point that was briefly raised in the letters by Soares et al and Iruela-Arispa and Graubert¹ is the role of progesterone in VEGF regulation. The role of progesterone in the regulation of VEGF has not been well addressed. A limited number of studies show that VEGF is under progestin regulation in both uterine and in a subset of breast cancer cells.^{5,12,16} It seems that progesterone induction of VEGF is also under strict cell type control.⁵ We have determined that VEGF is under progestin regulation in T47-D cells,⁵ and similar induction can be seen at both the message and protein level in BT-474 cells, but not in MCF-7 cells or in ZR-75 cells (our unpublished observation). This induction was not seen even after priming MCF-7 or ZR-75 cell with estrogen (10–9 mol/L for 48 hours) to up-regulate progestin response (our unpublished observations). Clearly, progesterone control of VEGF also seems to follow the same trend as estrogen control of VEGF, ie, it is cell type-dependent, and perhaps the progesterone responsive cells have either gained or lost a function that is important in order for them to respond to the hormone. This would be particularly insidious in occult tumors where progesterone may be able to drive the production of VEGF in selected cells, giving them a growth advantage over others, and leading to tumor expansion.

In conclusion, VEGF induction by steroid hormones appears to be tissue and cell type-dependent. Most evidence suggests that ER is required for estrogen response but that the receptor by itself is not sufficient. Similarly, progestin response seems to depend on progesterone receptor but the receptor does not seem to be sufficient for such an induction either. It is now important to further analyze the effects of hormones on expression of VEGF *in vivo* in different cells and tissues to gain a better understanding of the role of sex steroid hormones on VEGF regulation. While some data on uterine cells is available in this respect,^{11,12,14} data in different cells of the mammary gland is lacking. Studies need to be performed to identify biological importance of VEGF during tissue development, and to determine whether there is any heterogeneity within the mammary cells for hormone responsiveness with respect to VEGF induction. It is possible that not all mammary gland cells express VEGF in response to hormonal stimulation, and this may also be reflected in the fact that only selected mammary tumor cells respond to estrogen or progesterone *in vitro*. The need for *in vivo* data is important to understand the role of hormone-induced VEGF in progression of neoplastic disease to target anti-VEGF treatment effectively.

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