# Talking the talk: the role of VEGF proteins in cell signaling

Sandra M. Leal and Wendi S. Neckameyer

**Vascular endothelial growth factors (VEGFs) stimulate endothelial cell growth through interactions with their tyrosine kinase receptors to stimulate intracellular signaling events. This culminates in the expression of specific gene products that induce a cellular response in numerous physiological processes, including hematopoeisis, oncogenesis and embryogenesis. The primordial function of VEGF can be revealed by studying VEGF-mediated signaling pathways in the powerful and tractable model system, *Drosophila melanogaster*, which has proved invaluable in furthering our understanding of conserved developmental themes.**

In mammals, secreted vascular endothelial growth factors [VEGF-A, VEGF-B, VEGF-C, VEGF-D and placenta growth factor (PIGF)] stimulate endothelial cell growth through interactions with VEGF receptors (VEGFR-1, VEGFR-2, VEGFR-3 and neuropilin-1), a family of receptor tyrosine kinases (RTKs). VEGF-bound, activated receptors stimulate intracellular signaling events mediated through the mitogen-activated protein kinase (MAPK) cascade. This culminates in the expression of specific gene products that induce a cellular response. Circulating VEGF is also a potent chemoattractant for the recruitment of monocytes to sites of immune responses or inflammation. By acting as both a mitogen and a cell recruiter, VEGF regulates a wide range of physiological processes that include angiogenesis, vascularization, hematopoeisis, embryogenesis, wound healing and oncogenesis.

## How did the VEGF signaling pathway evolve to coordinate these diverse processes in mammals?

Cho *et al.* [1] studied VEGF and VEGFR function in *Drosophila melanogaster*. *Drosophila* is a powerful model system in which a broad range of molecular, genetic and cell-biological techniques can be applied to dissect the roles of signaling pathway components. Flies have their own repertoire of homologous genes that encode VEGFs (*Vegf27Ca*, *Vegf27Cb* and *Vegf17E*) and VEGFR (*stasis*). However, unlike their vertebrate counterparts, flies lack endothelial cells and the vasculature of a closed circulatory system. Thus, by studying VEGF signaling pathways that evolved in *Drosophila*, the primordial function of VEGF can be investigated.

Cho *et al.* [1] have identified a conserved function for the VEGF signaling pathway in hematopoiesis. In the *Drosophila* embryo, VEGF guides the migrational fates of hemocytes (blood cells). The staged spatial distribution of hemocytes during early and late embryonic development, described as generated 'waves of hematopoiesis' by Traver and Zon [2], is absolutely dependent upon VEGF activity. This was shown by simultaneously inactivating all the genes that encode VEGFs in the embryo via RNA interference, which resulted in limited expansion and placement of hemocytes. Similar studies in mammals show that loss of VEGF-A activity in the yolk-sac endoderm inhibits hematopoietic and endothelial differentiation [3], resulting in lethality. The differentiating properties of VEGF in *Drosophila* have not yet been firmly identified. However, the positional fate of cells is absolutely determined by VEGF in a hierarchical manner and hypothetically, once established in 'time and space', the cells receive morphogenic inputs from their local environment. Likewise, VEGFR mutants generated in both flies and mammals display similar cellular migrational defects. Targeted disruption of the *Vegfr2* gene in mice led to migrational defects of mesodermal progenitor cells and a concomitant loss of their endothelial and blood-cell daughters that compose yolk-sac blood islands [4]. In *Drosophila* VEGFR mutants, migration of blood cells from the caudal margin to the posterior tail region (embryonic stage 11) is halted. Taking the analyses one step further, inactivation of MAPK in the intact embryo may phenocopy *vegf* and *vegfr* mutants. Complete inactivation of MAPK is not possible in *Drosophila* owing to maternal and zygotic expression [1]. However, MAPK activity was significantly suppressed in *Drosophila* VEGFR mutants [1]. Finally, ectopic misexpression of VEGF in the developing foregut and other tissues misdirected blood cells within the axis of the embryo to more anteroventral positions.

## VEGF signaling coordinates embryonic cell movements across diverse species

Taken together, these data strongly support the observation that VEGF signaling pathways intimately coordinate embryonic blood cell movements in both mammals and *Drosophila*. The conservation of this relationship has also been observed in zebrafish embryos [5]; simultaneous inactivation of zebrafish VEGF and the VEGFR (*znrp1*) employing morpholinos (antisense oligonucleotides) inhibited blood cell circulation. Whilst much attention has been focused on VEGF-regulated blood-cell migration, neuronal recruitment could also be regulated, either directly or indirectly, by VEGF. In zebrafish, *Znrp1* transcripts were detected in both vascular and neuronal tissues. In the adult songbird brain, testosterone-induced recruitment of neurons requires a rapid burst of VEGF and VEGFR production to induce angiogenesis. The newly developed and expanded vasculature produces brain-derived neurotrophic factor (BDGF), which, in turn, recruits new neurons to the higher vocal center of the brain [6].

## VEGF is one of many signaling molecules in the guidance of conserved developmental pathways

In their comprehensive review, Traver and Zon [2] refer to the common theme of blood development as 'walking the walk'. We wish to take this one step further and suggest that the common developmental scheme adopted by VEGF signaling components be described as 'talking the talk'. It is apparent that as a collective group, many signaling proteins, referred to as 'generic regulatory molecules' [7], have adopted conserved talking (recruiting) strategies to guide cells to their proper places in the embryonic environment. Once in place, these progenitor cells undergo complex differentiation processes. As an example of common talking schemes shared by other signaling proteins, it has been shown in *Drosophila* that male-specific fibroblast growth factor (FGF) recruits mesodermal cells expressing FGF receptors to the genital imaginal disc, which develops into part of the internal

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genitalia [7]. The *Drosophila* transforming growth factor-α (TGF-α) homolog Spitz, a ligand for the epidermal growth factor receptor, functions to recruit cells into the developing retina [8]. Currently, many new signaling proteins are being identified from the *Drosophila* genome sequence [9] that include RTKs of the insulin-like growth factor-I/insulin family, a TGF-β ligand, Wnt ligands that regulate cell polarization and migration, and members of the Toll pathway which establish the embryonic dorso–ventral axis. In conclusion, signaling proteins, including VEGF, are proving instrumental in guiding developmental pathways through cell–cell communication. The emergence of new signaling factors makes this an exciting time for researchers to explore conserved developmental themes, whether they are classified as 'walks' or 'talks', employed by many species to promote appropriate developmental processes.

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**Sandra M. Leal**
**Wendi S. Neckameyer***
Dept of Pharmacological and Physiological Sciences, Saint Louis University School of Medicine, 1402 South Grand Boulevard, St Louis, MO 63104, USA.
*e-mail: neckamws@slu.edu


Meeting Report

# Sex, hormones and the cardiovascular system

Stacey A. Fannon and Sherry A. Marts

**The meeting 'Sex Differences in Cardiovascular Health and Disease' was held in Madison, WI USA, on 24 July 2002.**

Against the backdrop of the terminated Women's Health Initiative (WHI) trial to evaluate the benefits of estrogen plus progestin replacement therapy [1], 'Sex Differences in Cardiovascular Health and Disease' – a scientific advisory meeting organized by the Society for Women's Health Research – got underway. One of the meeting's goals was to address the molecular basis underlying hormonal effects on the cardiovascular system.

## Ligands, receptors, genomes and physiology – an integrated approach

Virginia Miller (Mayo Clinic, Rochester, MN, USA) emphasized the need for an integrated approach to studying effects of sex hormones on physiology. This point was brought home only too well by the results of the WHI, which showed that a combination of estrogen and medroxyprogesterone, whilst decreasing the risk of colorectal cancer, increases the risk of breast cancer and thrombotic events. Miller stressed the need to understand fully the complex interactions between ligands, receptors and genomes before hormones or their analogs are used as potential therapeutics.

Crucial to this understanding is the fact that hormone receptors are distributed heterogeneously throughout the body. Endogenous estrogens, synthetic receptor modulators (e.g. raloxifene), and naturally occurring phytoestrogens obtained through the diet, for example, have varying effects on target cells and tissues. These variations might reflect differential expression of receptor isoforms and/or different ligand-receptor affinities. In addition, single- and multiple-nucleotide polymorphisms within the population add an extra level of complexity, and could explain, for example, why estrogen elevates plasma endothelin-1 in some recipients, but decreases it in others. 'It would be interesting,' said Miller, 'to go to the WHI and [ask] which women developed different types of cancer or cardiovascular events and what were the polymorphisms in their estrogen receptors.'

Elucidating the molecular mechanisms of hormonal action is essential to the integrated approach. Traditionally steroid effects have been viewed as genomic (i.e. mediated through transcriptional activation), but more recently evidence has grown in support of non-genomic actions.

## Molecular effects on cardiovascular smooth muscle

In addition to the actions of estrogen on the endothelium, it has been known for some time that estrogen can relax denuded coronary arteries [2]. Richard White (Medical College of Georgia, Augusta, GA, USA) reported that 17β-estradiol, but not the biologically inert 17α- isoform, relaxes the smooth muscle from porcine coronary arteries, confirming that this action is not caused by some non-specific steroidal action, as had been previously suggested. So, what is the molecular basis for this action?

To answer this question, White tested if estrogen activates $K^+$ channels, because increasing $K^+$ efflux is one of the most efficient means of hyperpolarizing (and therefore relaxing) smooth muscle cells. Patch clamp recordings show that 17β-estradiol, even at concentrations as low as 1 nM, elicits a current through the cell membrane that can be inhibited by the $K^+$-channel blocker, IBTX. The electrophysiological properties of this estrogen-activated channel were found to be consistent with only one known $K^+$ channel – the $Ca^{2+}$-activated large conductance channel, or big conductance channel ($BK_{Ca}$). Similar recordings made

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