Gene Regulation During the Development of Embryonic Vascular Endothelium – Reflections of an Irish Postdoc in Japan

To the Editor:

Having spent almost 1 year as a member of the Genetics Division at the National Cancer Center, here is a summary of some of the most salient observations and experiences during my visit.

Following my previous research experience in the field of prostate cancer, I now found myself immersed in a novel and exciting field of research, namely vasculogenesis/angiogenesis. Initially encouraged by the earlier success of past and present researchers at the Genetics Division, I was furthermore pleasantly surprised upon arrival in the laboratory by the flexible and open-minded disposition tendered towards my views and project proposals. Japan has produced a number of independent research groups excelling in the field of angiogenesis/vasculogenesis, led by scientists such as Dr Shibuya (Tokyo University) and Dr Nishikawa (Kyoto University), to name only two.

The Genetics Division is unique in its strong emphasis on cancer research, including gene therapy for cancer, which is combined with a growing interest in developmental biology, particularly Dr Ochiya’s section for studies of metastasis. Since both disciplines are inextricably linked, this combination of research interests provides a valuable mutual nexus. Thus arriving into this environment I benefited from both areas of expertise. During my year I focused on the process of vasculogenesis (endothelial cell differentiation) during embryonic development. The cells of all blood lineages and all endothelial cells are believed to arise from committed progenitors descendant from a common bipotential hemangioblast. The aim of this project was to understand how commitment to specific lineages is programmed and cell-specific patterns of gene expression are established.

As a pretext to identifying the factors required for endothelial and hematopoietic differentiation and their mechanisms for programming lineage commitment, the precursor cells must be isolated. We have developed a strategy to attempt to identify these precursor cells (angioblast/bipotential hemangioblast) by labeling them with EGFP. Since flt-1 is expressed by the hemangioblast and one of the earliest endothelial-specific genes expressed during development, we rationalized that the promoter for the flt-1 gene would be a suitable candidate for targeting EGFP expression to early and pre-differentiated endothelium.

The endothelial-specific cell adhesion molecule PECAM-1 is strongly expressed in the endothelial lineage from an early stage during embryoid body (EB) development. Immunostaining experiments revealed that EGFP expression driven by the 2.2 kb flt-1 promoter strongly co-localizes with PECAM-1 in EBs derived from FLT.EGFP-transfected ES cells, indicating that the 2.2 kb flt-1 promoter is endothelial specific. Moreover, the consistent co-localization of PECAM-1 and EGFP in EBs displaying a range of vascular maturity indicates that there is significant overlap in their expression profiles. Very few EGFP-positive cells (<5%) did not express PECAM-1, which is an indication that expression of both genes is initially temporally matched. Since we know that PECAM-1 expression is an early event during endothelial differentiation, we can infer that EGFP is also expressed early in EB development. Indeed, our RT-PCR data confirm that FLT.EGFP is expressed from an early stage in EB development.

The alternatively spliced sflt-1 (soluble flt-1 – lacking the membrane proximal Ig-like domain), the membrane spanning polypeptide and the entire intracellular tyrosine kinase-containing region can inhibit mitogenic responses to VEGF in cultured cells by directly sequestering VEGF. This does it in a dominant negative fashion by heterodimerizing with the extracellular ligand-binding region of the membrane spanning flt-1 and flk-1, thereby preventing receptor tyrosine trans-phosphorylation and activation of downstream signal transduction. Recently overexpressed sflt-1 was used to inhibit tumor growth in vivo, impeding metastatic nodule development and extending host survival.

It has been demonstrated that flt-1 attenuates hemangioblast proliferation, a finding that is compatible with the abnormal assembly of endothelial cells into vascular channels observed in flt-1/− mice. However, deletion of the tyrosine kinase domain of the full-length flt-1 appears to have little effect upon either embryonic development or angiogenesis in mice. Collectively, these studies suggest that the primary function of flt-1 is simply to sequester VEGF family members in a dominant-negative manner. However, the individual contributions made by each of the flt-1 splice variants towards this phenotype remain unresolved. We designed variant-specific PCR primers located within the 5′ unique sequence of each cDNA and demonstrated that sflt-1 is expression prior to full-length flt-1 with subsequent increase in full-length flt-1:sflt-1 ratio during EB development. Hence it appears that the earliest role of flt-1 is to repress VEGF signaling via sflt-1. Later, this effect may be attenuated when expression of full-length flt-1 is upregulated. It is worth noting that in cells lacking flk-1 expression, flt-1 activation by VEGF does not induce cell proliferation, but flk-1 activation by VEGF in flt-1-negative cells does.

By using the 2.2 kb flt-1 promoter we now have a means to target ectopic gene expression to differentiating endothelium to modify or block this differentiation program and, additionally, the means to identify transcription factors that are important for vasculogenesis.

One outstanding feature of my period at the National Cancer Center centered on the weekly meetings. As a solitary
foreigner amongst more than 30 Japanese scientists, I was greatly impressed with the decision to conduct both the weekly journal club and laboratory data seminars in English instead of the traditional Japanese. Remarkably, each member of the Genetics Division bore this encumbrance with stoicism and succeeded in communicating both exciting research data and pertinent published research reports effectively through a foreign language.

A great asset of the National Cancer Center is its collective size and diversity of research activities, a feature that emerges at the monthly seminars. The availability of specialized microscopes, flow cytometry stations and other items of laboratory hardware is superb. Moreover, where not available in the Genetics Division, access to equipment in other Divisions was readily provided.

In conclusion, the National Cancer Center provides a highly stimulating and pleasurable environment for a foreign research scientist. During my period of research at the Genetics Division I have made valuable contacts and forged collaborations, which I hope will continue for many years.

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