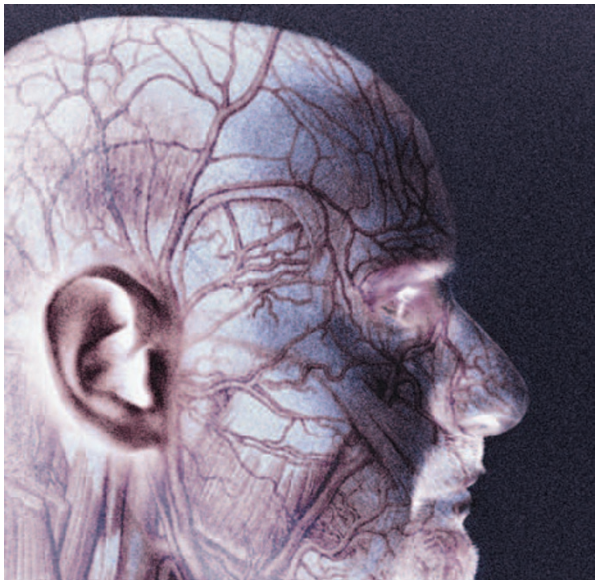
ANGIOGENESIS

Initiation

It is unclear how vascular endothelial growth factor (VEGF) expression controls the complex cellular interactions that occur during angiogenesis in adult tissues. In their recent *Cell* paper, Eli Keshet and colleagues have demonstrated that bone-marrow-derived cells contribute to angiogenesis by a paracrine process that is initiated by VEGF.

The authors constructed a transgenic mouse model in which transcription of a *VEGF* transgene is switched on in either the liver or the heart by the removal of tetracycline from the animals' drinking water. Inducing expression of VEGF in these tissues led to the recruitment of circulating bone-marrow-derived cells, a process that was evident after 4 days and preceded the generation of new vessels. Verification that these cells were recruited from a circulating bone-marrow-derived population was achieved by transplanting the bone marrow of the transgenic mice with syngeneic bone-marrow-derived cells expressing green fluorescent protein or β-galactosidase. Notably, the bone-marrow-derived cells were not seen to incorporate into the existing tissue endothelium, indicating that these cells do not function as endothelial progenitors. Instead, the bone-marrow-derived cells adopted a perivascular localization. Isolation and analysis of these cells from both the liver and the heart indicated that they were predominantly haematopoietic in nature.

> "bone-marrow-derived cells contribute to angiogenesis by a paracrine process that is initiated by VEGF"



The authors hypothesized that VEGF expression might recruit and retain the bone-marrow-derived cells by inducing the expression of a chemokine. Analysis of the gene-expression profiles of the bone-marrow-derived cells indicated that almost all of them expressed the chemokine receptor CXCR4. Importantly, the CXCR4 ligand, CXCL12, is known to be central to the recruitment and retention of many cell types, including tumour cells, to various tissues. The authors established that expression of VEGF in the heart and liver induced expression of CXCL12 in cells (probably fibroblasts or smooth-muscle cells) that surround the blood vessels. The authors also demonstrated the perivascular expression of CXCL12 in a prostate tumour model of angiogenesis. It is not yet clear how VEGF induces the expression of CXCL12 in perivascular cells.

VEGF expression is required to maintain the presence of the bone-marrow-derived cells because switching off VEGF expression in either the heart or the liver resulted in the loss of these cells. Furthermore, inhibiting the binding of CXCR4 to CXCL12 in the presence of VEGF also inhibited bone-marrow-derived-cell recruitment. Expression of CXCL12 alone failed to recruit bone-marrow-derived-cells, but did retain these cells when VEGF expression was switched off. So, VEGF and CXCL12 effectively combine to recruit and retain circulating bone-marrow-derived cells.

Finally, the authors analysed whether the recruitment of these cells had any effect on angiogenesis. *In vitro* vessel-sprouting assays demonstrated that the bone-marrow-derived cells were able to stimulate vessel growth through a paracrine mechanism, and *in vivo* assays demonstrated that the bone-marrow-derived cells stimulated the proliferation of the resident vascular endothelial cells in the liver and heart tissue. This was inhibited when retention of the bone-marrow-derived cells was inhibited by a CXCR4 inhibitor.

The authors conclude that disruption of the CXCL12–CXCR4 interaction is a possible target for anti-angiogenic therapy.

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ORIGINAL RESEARCH PAPER Grunewald, M. *et al.* VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. *Cell* **124**, 175–189 (2006)