CHAPTER 3

VEGF Gene Regulation

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Abstract

VEGF is best known for its angiogenic properties. Not only does it promote the growth of new blood vessels during embryonic development, it is also important in the adult, where it plays a role in maintaining an adequate supply of oxygen and nutrients to most tissues. VEGF gene regulation is controlled by different signalling pathways depending on the context in which it is expressed. Best understood is the induction of VEGF expression by hypoxia in neonates and adults, which represents an adaptive response to metabolic stress. In contrast, the mechanisms that control VEGF expression during embryonic development are currently less clear.

Key Messages

- VEGF is a multifunctional molecule that is regulated by numerous different signalling pathways.
- VEGF expression is induced by hypoxia.
- Hypoxia stimulates VEGF transcription by increasing HIF activity.
- Hypoxia stimulates VEGF translation by increasing mRNA stability.
- VEGF gene expression is also controlled by hypoxia-independent mechanisms, in particular during embryogenesis.

Introduction

More than half a century ago, Michaelson used an ink perfusion technique to visualize the developing retinal vasculature and noticed that capillary growth near veins was much more vigorous than near arteries. He proposed the existence of a vasoformative molecule termed factor X, which is (a) produced by extra-vascular tissue, (b) distributed in a gradient and (c) antagonized by oxygen. As we now know, these criteria are fulfilled by the vascular endothelial growth factor VEGF, also known as VEGFA. A team lead by Eli Keshet was the first to propose that VEGF could be the long elusive factor X that mediates hypoxia-induced vascular growth. This was based on the knowledge that VEGF had already been shown to promote the growth of blood vessels, and on the observation that VEGF mRNA dramatically increases under hypoxic conditions in various cell lines. In addition, it was found that VEGF levels were increased in the hypoxic centre of tumours, suggesting that this factor could mediate the growth of new vessels into tumours. This had important clinical implications, because it was known since the early seventies that tumour growth requires sprouting of new vessels from preexisting vessels, a process known as angiogenesis. The discovery of an angiogenic factor in tumours provided for the first time a molecular target for anti-angiogenesis therapy in the fight against cancer, triggering massive research on VEGF. In 1996 two teams simultaneously reported the

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genetic deletion of \( VEGF \) in mice. Both groups found that the inactivation of just one \( VEGF \) allele caused early embryonic lethality. This was unexpected and made further experiments technically challenging, as no heterozygous founder mice could be created. It also dramatically illustrated the importance of correct \( VEGF \) dosage during embryogenesis. Subsequent research has uncovered a multitude of mechanisms that tightly control \( VEGF \) dosage and its biological activity.

**VEGF Gene Regulation through the Hypoxia Response Element**

The most prominent stimulus for \( VEGF \) expression is hypoxia. In hypoxic tissue, \( VEGF \) is upregulated, and this stimulates blood vessel growth. Increased blood supply then alleviates the hypoxia, turning \( VEGF \) expression off again. This simple negative feedback loop ensures that supply and demand of oxygen in tissue are always adequately matched. But how does this process work at a molecular level? A short sequence in the 5' flanking region of the \( VEGF \) gene is important for \( VEGF \) induction by hypoxia. This sequence element, termed hypoxia response element (HRE), was initially discovered as an enhancer element within the erythropoietin (EPO) gene. The element is also present in many other hypoxia inducible genes and is a binding site for the transcription factor hypoxia-inducible factor 1 (HIF1). HIF1 is a heterodimer consisting of an alpha and beta subunit, both of which are basic helix-loop-helix PAS domain proteins. The beta subunit is known as the aryl hydrocarbon receptor nuclear translocator (ARNT) and is constitutively expressed, whereas the alpha subunit, HIF1A, is regulated by hypoxia. Although the mRNA encoding HIF1A increases in hypoxic cells, the dominant mechanism of HIF1A regulation is post-translational (Fig. 1). Under normoxic

![Figure 1. HIF mediated VEGF transcription. Under normoxic conditions, hypoxia inducible factor (HIF) is hydroxylated by prolyl hydroxylases (PHDs). This modification facilitates binding of the von Hippel Lindau protein (VHL), resulting in ubiquitination and rapid degradation of HIF. At low oxygen concentration, hydroxylation via PHDs becomes less efficient, resulting in HIF accumulation and HIF binding to a hypoxia response element (HRE) in the VEGF promoter.](image-url)