Id1 Regulation of Angiogenesis


Rhoda M. Alani

The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland

Inhibitor of differentiation (Id) proteins are primarily involved in regulating cellular differentiation. They are dominant-negative helix-loop-helix (HLH) transcription factors. Normally, basic helix-loop-helix (bHLH) transcription factors bind DNA as dimers primarily to E-boxes and often regulate genes important for differentiation. Id proteins lack the basic domain and therefore they can’t bind DNA. Thus, Id can be taken to mean inhibitor of DNA-binding or inhibitor of differentiation. Over the past decade, Id proteins have been determined to regulate many processes involved in tumorigenesis including tumor angiogenesis, tissue invasion by regulating MMPs and CXCR4, cellular proliferation primarily by regulating the Rb pathway and p16 as well as ETS transcription factors, and dedifferentiation or anaplasia by regulating the tissue-specific HLH transcription factors (reviewed in Ref. 1).

Id1, -2 and -3 are expressed ubiquitously, while Id4 is expressed in neurons. In adults, most expression is in proliferating stem cells. Id1 KOs have no obvious phenotype. Id2 KOs lack NK cells, Peyers patches, and Langerhans cells, and have defective spermatogenesis. Id3 KOs have altered humoral immunity.

Id genes made headlines in October 1999 when work from Robert Benezra’s lab demonstrated that mice that lacked both Id1 and 1d3 had loss of viability with defects in neurogenesis and angiogenesis in the brain. In xenograft tumor models, heterozygous double knockouts were unable to support growth of any of three types of tumor cells due to defective angiogenesis. This manuscript suggested that inhibiting Id gene function would be an effective means of targeting a large variety of tumors therapeutically.

How do Id genes affect the angiogenic process? In order to answer this question it is necessary to look at the downstream effectors of this transcriptional regulatory protein. What are downstream effectors of Id genes? Using PCR-based subtractive hybridization, genes were identified that are selectively expressed or downregulated in Id1 knockout compared to Id1 wild type mouse embryo fibroblasts. Genes repressed by Id1 are upregulated in null cells and include thrombospondin 1 (Tsp1), β3 integrin, fibronectin, and other adhesion molecules. The upregulation of Tsp1 was confirmed by quantitative RT-PCR. In cells transfected with a Tsp1 promoter/luciferase reporter construct, addition of Id1 results in reduction in luciferase activity. An attempt was made to map the Id1 responsive areas in the promoter, but even deleting down to 160 bp, there was still some repression, suggesting that it is an indirect effect. Conditioned medium (CM) from cell cultures derived from Id1 KOs caused less endothelial cell migration than CM from wild type cell cultures and this was reversed by antibodies to Tsp1. Matrigel implant assays were done in which VEGF- or FGF2-loaded matrigel was implanted in Id1 KOs or wild type mice. The KOs had less angiogenesis and there was Tsp1 staining around vessels.
These data suggest that Tsp1 is a downstream effector of Id1; the effects of Id1 on Tsp1 are indirect and Tsp1 is a major determinant of Id effects on angiogenesis.

Id made headlines again in November 2001, when work from Shahin Rafii’s lab showed that tumor growth and angiogenesis could be re-established in Id KOs by bone marrow transplantation. How does the bone marrow do this? There is evidence to suggest that these cells are endothelial cells. This is the pathway felt to be involved in endothelial cell development from hemangioblasts. However, is the tumor xenograft model representative of human cancer? Autochthonous tumor systems in which a tumor develops in a mouse is more relevant. One such model system is two step tumor induction of skin cancer in mice using DMBA/TPA treatment. In this setting, mice develop papillomas which ultimately develop into invasive squamous cell carcinomas. Id1 KO mice treated with DMBA and TPA developed more papillomas than wild type mice and there was no difference in angiogenesis. This data was counter to the previous results using tumor xenografts which made us question whether Id1 KO mice also possessed a defect in tumor surveillance. Since γδ T cells play an important role in skin tumor surveillance and receptor KO mice have increased skin cancer susceptibility, γδ T cells were assessed in the skin of Id1 KO mice and found to be markedly decreased. Is this because they are not being made in the thymus or are they just not getting out to where they should be? The embryonic thymus was normal; there was just a difference in γδ T cells in skin. Is there a defect in migration or premature death of γδ T cells? Thymocytes from wild type or Id1 KO mice were injected into Rag KO mice and while wild type thymocytes could produce γδ T cells in skin, those from Id1 KOs could not, suggesting either a migration defect or cell survival defect in Id1 KO thymocytes. The Id1 KO thymocytes had reduced migration to SDF-1 and this was determined to be due to reduced expression of CXCR4 in Id1 KO thymocytes.

While these studies have identified a new Id-mediated pathway involving CXCR4, this work further points out an important difference between xenograft and autochthonous tumor models. Since tumor xenografts are unable to grow in Id1 KO mice due to defects in tumor-associated angiogenesis while autochthonous tumors show no angiogenic defects in these same mice, we suggest that the sudden appearance of a large load of cells in xenograft models results in a large release of angiogenic factors stimulating angiogenesis that is Id-dependent and bone-marrow derived. In contrast, the gradual growth of tumors in autochthonous tumor models is accompanied by slow induction of angiogenesis as tumors go from in situ to invasive disease. Angiogenic factors rise slowly and oxygen and glucose are slowly depleted and results in angiogenesis that is Id-independent and derived from neighboring vessels.

A recent study investigated the source of endothelial cells in tumors that developed in patients who had had bone marrow transplants. Tumor biopsies from six patients that had received transplant cells derived from a member of the opposite sex were examined with q-fish and immunohistochemistry to determine the origin of endothelial cells. It was found that 4-12% of endothelial cells in tumors were derived from the bone marrow. Tumor types were unusual in that there were no epithelial tumors, but regardless, it suggests that the bone marrow can contribute, but only a very small component of the endothelial cells are derived from the bone marrow. We suggest that bone marrow angiogenesis is essentially Id-dependent angiogenesis. There is acute, severe hypoxia, which results in recruitment of cells from the bone marrow, and then gradually there is remodeling and the bone marrow components are decreased. This suggests that it might be possible to use bone marrow stem cells as therapeutic agents in hypoxic disease.
Question

1. What is the mechanism for Id-dependent bone marrow associated angiogenesis?
   CXCR4 may be involved and Id regulation of CXCR4 may be important for bone marrow cells, the hemangioblasts and precursor cells, to get out of the bone marrow. Studies are currently underway to test this hypothesis.

References