Integrins and Extracellular Matrix


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Regulatory signals come from the cellular compartment (tumor cells, stromal cells, inflammatory cells, and vascular cells) and the acellular compartment. The acellular compartment is the extracellular matrix (ECM). There are cryptic sites in ECM that when exposed can participate in the regulation of angiogenesis. Three groups of molecules are involved: integrins, matrix metalloproteinases (MMPs), and ECM components such as collagens or laminin. Remodeling of the ECM by MMPs can expose cryptic sites that interact with integrins.

To develop reagents that could recognize proteolyzed or denatured forms of collagen, but not the native triple helical form, subtractive immunization was used to generate monoclonal antibodies. One antibody, HUIV26, recognizes a cryptic site in collagen IV and was used to stain normal skin and biopsies from malignant melanoma. Exposure of the HUIV26 cryptic epitope was observed around tumor blood vessels and associated with the invasive fronts of tumors as they penetrated epithelial basement membranes and little or no exposure as seen in normal skin. This was also the case in biopsies from several other tumor types. Systemic injections of HUIV26 inhibited FGF2-induced angiogenesis in chick chorioallantoic membranes (CAMs), and the antibody also blocked cellular adhesion and migration.

How does this information get transferred to the cells? HUIV26 was capable of inhibiting purified αvβ3 binding to denatured collagen, but had no effect on α2β1 binding, suggesting that the cryptic site is recognized by αvβ3. This site is a non-RGD site. Does this site also play a role with regard to tumor cells? It was previously demonstrated that expression of αvβ3 by tumor cells gives them a growth advantage. M21 cells that express αvβ3 were compared to a variant of the cells that don’t express αvβ3, and it was found that the cells that express αvβ3 grew better in vivo, but not in vitro. This suggests that something in the in vivo environment is giving them a growth advantage. In a xenograft tumor model, the cells that lacked αvβ3 formed tumors with less vascular density than the tumors formed from cells that expressed αvβ3. Cs1 melanoma cells that lacked αvβ3 were transfected with β3 and put on chick CAM, and blood flow was measured. Tumors induced by cells expressing β3 had greater blood flow. Conditioned medium (CM) from cells that lack αvβ3 inhibited endothelial cell proliferation, inhibited angiogenesis in CAM assay, and inhibited tumor growth. Microarray analysis demonstrated many genes that were upregulated and many that were downregulated in αvβ3-negative cells. Thrombospondin-1 (Tsp-1) was upregulated and the CM was found to have high levels of Tsp-1. Members of the insulin-like growth factor binding protein (IGF-BP) family were also upregulated. Knock down of αvβ3 with siRNA in cells that express it resulted in upregulation of Tsp-1.
Cells were allowed to attach to vitronectin (αvβ3-mediated) or to triple helical collagen (β1-mediated), and when they were attached to vitronectin there was suppression of Tsp-1 expression. What happens if cells are plated on denatured collagen (exposes the cryptic site recognized by αvβ3, but also has β1 sites - so it is a mixed ligand) and then treated with LM609, which binds αvβ3? CM collected after 12 hours demonstrated upregulation of Tsp1 compared to controls. LM609 antibody was coated on a plate to drive a specific signal through αvβ3. Compared to cells plated on an anti-β1 antibody, there was suppression of Tsp1 levels in the CM of cells in which signaling was driven through αvβ3. So ligation or binding of αvβ3 results in suppression of a known angiogenesis inhibitor, Tsp1.

The results were similar for IGFBP4. Knockout of αvβ3 resulted in upregulation of IGFBP4. Treatment of cells plated on denatured collagen with LM609 resulted in upregulation of IGFBP4. M21 melanoma cells were implanted on CAM and the embryo was treated with LM609 or an isotype-matched control antibody. Immunohistochemistry showed upregulation of IGFBP4 in tumor cells from LM609-treated embryos. In another model, ng quantities of IGFBP4 inhibited FGF2-induced angiogenesis.

When cells were plated on denatured collagen in the presence of HUIV26 (blocks cryptic site), there was upregulation of Tsp1 and IGFBP4. In endothelial cells, the results were similar. If you treat denatured collagen with HUIV26 and plate HUVECs, there is upregulation of Tsp1 and IGFBP4.

We tested the effect of LM609 in a nude mouse model. M21 human melanoma cells were injected into mice and then mice were treated with LM609 or an isotype-matched control antibody. After 7 days, the tumors in mice treated with LM609 were almost avascular. This may be due to LM609 binding to αvβ3 on tumor cells and indirectly inhibiting angiogenesis by increased production of Tsp1 and IGFBP4.

The working hypothesis is that αvβ3 is expressed on tumor cells (high in invasive malignant melanoma), and a number of MMPs are being produced by tumors, stroma, and inflammatory cells. The MMPs can degrade ECM and expose cryptic epitopes, and αvβ3 on tumor cells can then recognize and bind to these cryptic epitopes, drive a signal through FAK and ultimately through Ras (Ras activates PI3 kinase, Rou DGS, Raf MAP kinase). Activation of the MAP kinase pathway can lead to activation of ERK which phosphorylates and activates cMyc, which acts as a repressor of Tsp1. Alternatively, the PI3 kinase pathway is activated, which can activate cMyc and repress Tsp1. So one of these pathways may be involved in the ability of αvβ3 on tumor cells to suppress these endogenous inhibitors of angiogenesis, and if this is blocked by blocking αvβ3 or blocking the cellular access to the cryptic epitopes, the repression of Tsp1 is removed, and thereby Tsp1 is increased and suppresses angiogenesis.

Questions

1. Do you have any data on IGFBP3, because it is commonly considered a proapoptotic agent?
   IGFBP3 was upregulated at the mRNA level, but in the CM there was not an increase in IGFBP3 protein.

2. The increase in IGFBPs would have an effect on local IGF1 levels. Have you looked at that?
   Not yet, but it may be one part of how the system is working.