

Chemistry and Molecular Biology Department and NIH Center for Protease Research
Candidate Seminar
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“Regulation of Tumor Progression by the Ligands of $\alpha 9\beta 1$ Integrin”

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Abstract

Integrins are the family of cell surface receptors that are involved in cell-cell and cell- extracellular matrix assembly, as well as in signal transduction during physiological and pathological processes. The signal transduction abilities of integrins are important in cell survival/death regulation leading to consideration of these receptors as attractive targets in several disorders, including cancer. Initiation and progression of this still deadly disease is dependent on a variety of circumstances including intensively growing vasculature known as pathological angiogenesis. Our investigations revealed that $\alpha 9\beta 1$ integrin is highly expressed on microvascular endothelial cells, as well as on certain tumoral cells such as glioma and breast cancer with intensity of expression correlated to increased malignancy. We found that this integrin is a receptor for thrombospondin-1 (TSP-1) and nerve growth factor (NGF), and their interaction with $\alpha 9\beta 1$ integrin leads to increased cell survival and proliferation. TSP-1 has an integrin binding site within the N-terminal domain (NoC1), which is released from the entire molecule by enzymatic digestion during tumorigenesis, such as progression of glioma. In the experimental models of angiogenesis in mice and quail embryos, recombinant NoC1 domain significantly increased neovascularization, and this process was inhibited by specific blockers of $\alpha 9\beta 1$ integrin. NGF is also pathologically increased in malignant glioma, showing a high affinity for binding tumoral cells *in vitro* in an $\alpha 9\beta 1$ integrin-dependent manner. Astrocytoma cell lines expressing $\alpha 9\beta 1$ integrin following stimulation with NGF increased proliferation and migration ratios, whereas absence of this integrin on the cancer cell surface led to inhibition of cell proliferation by this growth factor that may be dependent on its interaction with another NGF receptor, p75^{NTR}. Binding of NoC1 or NGF to $\alpha 9\beta 1$ integrin on the cell surface results in activation of pro-survival MAPK Erk1/2 cell signaling pathway and phosphorylation of paxillin, an adaptor protein involved in regulation of focal adhesion.

Snake venom disintegrins were characterized as exogenous ligands for certain integrins, containing properties to block functions of these receptors in the physiology of the cell. We discovered new classes of these snake venom proteins, which have new biologically active motifs such as KTS and MLD. MLD-disintegrins appeared to be potent inhibitors of $\alpha 9\beta 1$ integrin in cell adhesion and ELISA assays. VLO5 is a member of these disintegrins and potently blocks NoC1- and NGF-induced angiogenesis and tumor progression *in vitro* and *in vivo*. Its angiostatic effect is correlated with ligation to the endothelial cells and induction of their apoptosis following binding to $\alpha 9\beta 1$ integrin. Similar effect was observed for glioblastoma cell lines that express $\alpha 9\beta 1$ integrin. Apoptosis induced by VLO5 was caspase-dependent and the mechanism of this process involves activation of the Fas pathway. VLO5 contains an SH3-binding domain motif in its C-terminus tail and is an exogenous Fas-ligand. Pro-apoptotic activity of VLO5 resulted in blocking of pathologically induced angiogenesis by NoC1, growth factors or developing glioma in mouse and quail models. Glioma growth in the embryonic quail chorioallantoic membrane (CAM) was also inhibited by VLO5, although this inhibition was significantly abolished by NGF. These results suggest that $\alpha 9\beta 1$ integrin is a multifunctional cell surface receptor, which is involved in transferring pro-survival and pro-apoptotic signals. Therefore, it participates in tumor progression by inducing angiogenesis and proliferation of tumoral cells, and it may also be an interesting target for development of an oncostatic therapy.