binds to an RGD motif within LAP and beta8-expressing cells adhere to LAP. Using co-culture assays with reporter cells that measure active TGF-beta, we find that alphavbeta8 can activate TGF-beta. Furthermore, we have used beta8-cytoplasmic truncation mutants to determine that the cytoplasmic domain of beta8 is not required for TGF-beta activation. Thus, the mechanism of alphavbeta8 mediated activation of TGF-beta is novel since the only other description of integrin mediated activation of TGF-beta (Munger, et al (Cell, 1999; 96(3), 319-328), involves a mechanism requiring the beta6-integrin cytoplasmic domain. Finally, we have identified that one function of integrin alphavbeta8 mediated activation of TGF-beta is to inhibit cell growth. Having previously identified b8 as a growth inhibitory molecule of the human airway, we now propose a plausible mechanism for alphavbeta8 mediated growth inhibition involving activation of TGF-beta.

1363

Role of αvβ6 integrin in growth and migration of oral squamous cell carcinoma.

Hui Xue[1], Amha Atakilit[2], Weimin Zhu[3], Dean Sheppard[2], Daniel M. Ramos[2], Robert Pytela[2], [1]Lung Biology Center, Department of Medicine, UCSF, 1001 Potrero Ave, Bldg 1, Rm 150, San Francisco, CA 94110, [2]UCSF, [3]Bayer Co.

The αvβ6 integrin is neo-expressed in a high percentage of SCCs (squamous cell carcinomas) derived from the oral mucosa. αvβ6 is known as an epithelial-cell specific fibronectin receptor, and also binds to and activates latent TGFβ1. To assess the function of αvβ6 in cultured SCCs, we used the cell line HSC-3, which is derived from a lymph node metastasis of human tongue cancer. This cell line is highly invasive in vitro and forms malignant tumors in nude mice. Cultured HSC-3 cells express αvβ6 on their surface, and tumors formed in nude mice are strongly stained by αvβ6-specific antibodies. A neutralizing monoclonal antibody to αvβ6, termed 10D5, blocked the adhesion of HSC-3 cells to the latency-associated peptide (LAP) of TGFβ1. Migration of HSC-3 cells on plastic substrates was substantially inhibited (approx. 50%) by the 10D5 antibody, but not by non-neutralizing anti- αvβ6 antibodies. Migration across fibronectin-coated filters was also inhibited by the 10D5 antibody. In addition, the anti-αvβ6 antibody almost completely prevented growth of HSC-3 cells in a three-dimensional collagen gel. To test whetherαvβ6 is involved in orthotopic SCC growth in vivo, HSC-3 cells were transorally injected into nude mice, together with the 10D5 antibody or non-neutralizing control antibodies. We found that the anti-αvβ6 antibody significantly inhibited HSC-3 tumor growth in vivo. These results point to a possible critical role of the αvβ6 integrin in controlling growth and invasion of human oral cancer cells.

1364

N-Myc and Bcl-2 Induce Changes in Adhesion Molecules and Susceptibility to Apoptosis in Human Neuroblastoma Cells

Cynthia M. van Golen[1], Daniel L. Noujaim[2], James S. Kim[2], Valerie P. Castle[2], Eva L. Feldman[2], [1]Department of Neurology, University of Michigan, 4414 Kresge III, Ann Arbor, MI 48109, [2]University of Michigan

Neuroblastoma (NBL) is a pediatric tumor arising from improper differentiation of neural crest cells. Expression of the N-Myc transcription factor is an effective prognostic factor in NBL; however, overexpression of N-Myc alone cannot increase tumorigenicity in some NBL cell lines. In nontumorigenic NBL cell lines, coexpression of both N-Myc and Bcl-2 can result in a tumorigenic phenotype, suggesting cooperativity between these molecules in promoting NBL tumorigenesis. SHEP NBL cells undergo apoptosis in response to serum withdrawal and hyperosmotic exposure. Apoptosis induced by either stressor is prevented by activation of the insulin-like growth factor (IGF) I receptor (IGF-IR) or Bcl-2 overexpression. Given the anti-apoptotic role of Bcl-2, we investigated the effects of coexpression of N-Myc and Bcl-2 on apoptosis. Expression of N-Myc alone enhanced apoptosis induced by either serum withdrawal or hyperosmotic exposure in SHEP cells and prevented rescue by IGF-I. The expression of Bcl-2 prevented apoptosis induced by either stressor regardless of the presence of N-Myc. N-Myc or Bcl-2 slightly enhanced IGF-IR expression in SHEP cells, yet activation of Akt, a protein which mediates growth factor induced survival, was decreased in SHEP/N-Myc cells but was unaffected in Bcl-2 expressing cells. Given the regulation of Akt by adhesion through the integrin linked kinase (ILK), we postulated a role for N-Myc in promoting tumorigenesis through modulation of integrin mediated signaling. ILK expression was decreased in SHEP/N-Myc cells while Bcl-2 enhanced ILK expression. N-Myc caused a decrease in ß1 integrin expression in serum withdrawal conditions, while addition of IGF-I enhanced ß1 integrin expression in SHEP/N-Myc cells. These data suggest that N-Myc may modulate apoptosis through regulation of IGF-IR and integrin mediated signaling. NIH NS36778, NS38849, the Program for Understanding Neurological Diseases (PFUND).

1365

Structural Analysis of Integrin Binding Peptides that Initiate Development

Jeffrey W. Norris[1], Melanie M. Tomczak[2], John H. Crowe[2], Richard L. Nuccitelli[3], [1]Section of Mol. and Cell. Biol., University of California, 1 Shields Ave., Davis, CA 95616, [2]University of California, [3]Molecular and Cellular Biology, University of California, Davis, 1 Shields Ave., Davis, CA 95616-8535

Fertilization of a *Xenopus laevis* egg is indicated by a calcium wave that originates at the point of sperm-egg fusion and propagates to the opposite pole of the egg. A peptide derived from the disintegrin domain of the sperm protein ADAM 16 can initiate this wave. This result has been controversial, because peptides in which the active sequence has been scrambled also initiate the calcium wave. Using the Chou-Fasman algorithm we predicted secondary structures of native ADAM 16 disintegrin domain, scrambled disintegrin domain, and alanine scanner mutants of the KTE active tripeptide. Comparison of the predicted structures leads us to conclude that the presence of beta turn at the KTE sequence is necessary for activity of the peptides. Fourier Transform Infrared Spectroscopy was used to analyze the structure of these peptides, and supports the interpretation of the prediction results.

1366

Identification of the binding site for fibrinogen recognition peptide within the I-domain of leukocyte integrin $\alpha_M\beta_2$

Valentin P. Yakubenko[1], Tatiana P. Ugarova[2], [1]Molecular Cardiology, Cleveland Clinic Foundation, 9500 Euclid ave, Cleveland, OH 44195, [2]Cleveland Clinic Foundation

The leukocyte integrin $\alpha_M\beta_2$ is a cell surface adhesion receptor for fibrinogen. The interaction between $\alpha_M\beta_2$ and fibrinogen is critically involved in leukocyte adhesion and migration during immune-inflammatory responses. The binding site for $\alpha_M\beta_2$ resides in the D domain of fibrinogen in its constituent γ-module. Within the γ-module, the sequence 383-395 (P2-C) has been implicated as a putative binding site for $\alpha_M\beta_2$. Previous studies have demonstrated that the P2-C peptide binds to the α_MI-domain of $\alpha_M\beta_2$ and four sequences in the I-domain, P^{147}-R^{152}, P^{201}-K^{217}, K^{245}FG and E^{253}-R^{261} were found to participate in P2-C binding. Further analyses of synthetic peptides corresponding to four identified segments showed that only two peptides, K^{245}-Y^{252} and E^{253}-R^{261} directly interacted with P2-C. In this study, we have localized amino acid residues within the α_MI-domain segment K^{245}-R^{261} responsible for P2-C recognition. Selected point mutations were introduced into this sequence in the recombinant I-domain and the ability of mutant proteins to bind to the immobilized P2-C peptide was tested. The choice of residues for mutational analyses was based on the observation that another related integrin, $\alpha_X\beta_2$, also binds to P2-C. We hypothesized that the residues involved in ligand docking within K^{245}-R^{261} should be: 1) conserved in the α_M and α_X I-domains and 2) exposed on the surface of the receptor. The six amino acid residues, K^{245}, F^{246}, G^{247}, D^{254}, P^{257}, D^{260} which meet these two requirements, were sequentially mutated. Mutations of three residues, F^{246}, D^{254} and P^{257}, caused significant loss of the I-domain binding to P2-C. These results indicate that residues within the sequence K^{245}-R^{261}, which forms a loop and a helix on a single face of the α_MI-domain, provide the ligand contact sites for binding of a short sequence of fibrinogen.

1367

Mapping of LPS-Binding Site on Human Leukocyte Integrin Beta-2 Subunit

Bobo W. Mok[1], John M. Luk[2], [1]Surgery, University of Hong Kong, NCB 203 Queen Mary Hospital, Pokfulam, HKSAR XXXXX Hong Kong, [2]University of Hong Kong

In innate immunity, bacterial lipopolysaccharide (LPS) is recognized as foreign particle by CD14 and toll-like receptor on the cell surface of human leukocytes. Engagement of these receptors initiates inflammatory cascades and clearance mechanisms such as bacterial phagocytosis in order to eradicate the invading pathogens. Leukocyte integrin Mac-1 and p150,95 have also been reported to bind LPS and trigger NF-kB translocation signaling. However, understanding of the LPS-integrin relationship remains largely unclear. Since LPS binds both Mac-1 and p150,95, it is probable that the integrin beta-2 common subunit CD18 entails a binding site for the LPS. The present study aims to delineate the LPS-binding site on integrin beta-2 subunit, and the findings are summarized as follows: 1) LPS is found to bind an isolated soluble form of CD18 in a dose-dependent manner, and the LPS-sCD18 interaction could be inhibited by a variety of bacterial LPS species; 2) using a panel of defined anti-CD18 mAbs in functional blocking assay, the LPS-recognition site was tentatively determined to locate between the distal I-like domain to the promixal part of the cystein-rich region. Further fine mapping is under investigation.(supported by grants from the University CRCG and HK Research Grants Council)