and HSV-2 infection in these three cells lines. Dual-label immunofluorescence revealed that Cx-43 expressing WB cells had Cx-43 present as plaques and ZO-1 distributed uniformly at the membrane contact points. Upon HSV-2 infection of WB cells, Cx-43 was lost from the membrane and ZO-1 was redistributed to the cytoplasm. In HSV-2 infected WB-aB1 and WB-a32/10 cells, Cx-43 and ZO-1 distribution remained similar to uninfected cells. Prior to and following infection, WB cells showed much lower levels of E-Cad in the cell membrane compared to the less permissive WB-a32/10 cell line. These results demonstrate that there is a change in distribution of communication-associated proteins in permissive cells following HSV-2 infection but [redacted] nissive mutant cells. In addition, the presence of E- [redacted] s to play a key role in the ability to produce an infection [redacted] This research was supported by NIH AREA grant R15 [redacted] GM, RW, and MK and WCU CAS student research grant to BD.)

788.3

Reduction of IQGAP1 increases insoluble VE-cadherin at endothelial adherens junctions

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The objectives of the present study were to determine if IQGAP1 associates with components of the endothelial adherens junction and if it affects endothelial barrier function. In human umbilical vein endothelial cells (HUVECs), soluble IQGAP1 associated with VE-cadherin and the catenins, β, γ, and α, but not N-cadherin. Gene silencing of IQGAP1 by transfection of small interfering RNA (siRNA) with oligofectamine maintained a higher endothelial electrical resistance in HUVECs as compared to transfection of a scrambled siRNA. Reduction of IQGAP1 induced an increase and a decrease, respectively, in the protein levels of VE-cadherin and N-cadherin. More VE-cadherin and less N-cadherin were associated with p120- and β-catenins in IQGAP1 knockdown cells. Furthermore, more insoluble (actin-associated) VE-cadherin was localized at intercellular junctions and less insoluble N-cadherin was present in the cell. These findings suggest that a reduction of IQGAP1 positively influences the endothelial barrier by increasing the protein level of VE-cadherin and the interaction of VE-cadherin with the actin cytoskeleton. The increased interaction with the catenins, β and p120, possibly resulting from the decrease in N-cadherin, may explain the increase in VE-cadherin (Supported by National Institutes of Health grants, HL-68079 (FLM) and HL-52406 (PAV)).

788.4

Differential regulation of VE-cadherin and N-cadherin levels by p120

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Endothelial cells express both VE-cadherin, which is endothelial specific, and N-cadherin. Our lab has shown that association of p120 with VE-cadherin is required for maintaining VE-cadherin levels and endothelial barrier integrity. We expected that N-cadherin levels, like VE-cadherin levels, would be regulated by p120. However, altering the amount of p120 in bovine pulmonary artery endothelial cells (BPAEC) using either adenovirus or siRNA did not affect N-cadherin levels. We have found that different endothelial cells express widely varying levels of N-cadherin. Increasing N-cadherin expression in human lung microvascular endothelial cells, which express little endogenous N-cadherin, resulted in increased junctional localization of N-cadherin as opposed to diffuse distribution across the surface of the cell, and also a decrease in VE-cadherin, which we hypothesized was due to competition for p120. This was confirmed by co-expressing p120 with N-cadherin, which prevented the decrease in VE-cadherin. We also found that high level expression of N-cadherin in BPAEC resulted in a decrease in barrier function as assessed by changes in electrical resistance and permeability to fluorescent-labeled albumin suggesting that N-cadherin-p120 interactions were not as effective as VE-cadherin-p120 interaction at supporting endothelial barrier function. This data illustrates that although p120 associates with both cadherins in endothelial cells, N-cadherin, unlike VE-cadherin, is not subject to regulation by p120 and does not promote endothelial barrier function.

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788.5

Effect of N-cadherin activation on Matrigel invasion and collagen I migration in melanoma cells

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Melanoma progression is associated with the downregulation of E-cadherin and the upregulation of N-cadherin, the so-called 'cadherin-switch'. The upregulation of N-cadherin has been hypothesized to potentiate pro-invasive and pro-migratory signaling in other cancers. This study examined the effect of the cyclic 14-mer peptide N-Ac-CHAVDINGHAVDIC-NH_2 which has previously been reported to activate N-cadherin (Skaper et al., Mol. Cell. Neurosci. 87(10):3398-3406, 2004). Two vertical growth phase melanoma cell lines, WM278 and A375, were grown in Tu2% media (4:1 mix $MCDB^{153}$:L15 with 2% serum, 5 μg/ml insulin, 2 mM $CaCl_2$). Treated cells were mixed with SW4 (50 μg/ml) for 30 min prior to assay. Invasion (24 hr) and migration (6 hr) assays were carried out with a 48-well modified Boyden chamber assay. Membranes were coated with 0.5 mg/ml Matrigel for invasion assay and 0.1 mg/ml rat tail collagen I for migration assay. Assays were run in Tu2% with or without SW4 (50 μg/ml) in the presence or absence of bFGF (10 ng/ml). Surprisingly, SW4 activation caused a substantial reduction in WM278 invasion (749±48 to 284±71 cells per 5-high power fields, ANOVA $p<0.001$). SW4 also caused a lesser reduction in WM278 collagen I migration (408±20 to 319±11, ANOVA $p<0.001$). Under the study conditions, the bFGF chemotactic effect was negligible. A375 invasion was low and did not change with SW4 (32±7 vs 34±8). A375 migratory was also low but increased with SW4 (113±12 to 154±8, $p<0.01$). The data suggest that under some conditions N-cadherin activation without homotypic, homophilic adhesion is capable of inhibiting melanoma invasion and migration.

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788.6

The hyaluronidase Hyal2 is a regulator of cell surface hyaluronan interactions

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HA may increase cell motility, facilitate cancer cell invasion and induce angiogenesis. Many of these effects depend on HA binding to specific receptors, mostly CD44, on the cell surface and on the presence of a pericellular HA-rich coat. We hypothesized that the ubiquitous glycosyl-phosphatidylinositol-anchored hyaluronidase Hyal2 could be one of the HA regulatory factors modifying cell response to HA.

In stable Hyal2 transfectants of the rat fibroblastic cell line BB16, Hyal2 was detected on the plasma membrane and in vesicles. The plasma membrane localization was confirmed by tranfection of a fluorescent Hyal2 chimera and by surface biotinylation experiments. The thickness of the pericellular hyaluronidase-sensitive coat was 1.22 ± 0.17 and 2.05 ± 0.45 ($n=52$; $p<0.0001$) in cells expressing high and low level of Hyal2, respectively. Hyal2 overexpression did not decrease the amount of endogenous cell-associated HA nor the amount of secreted HA, but it reduced the number of apparent ^{125}I-HA binding sites two-fold (Bmax: 0.17 ± 0.07 vs 0.07 ± 0.01 μg HA/10^6 cells; $n=7$; $p<0.05$) without significant changes in their dissociation constant. The level of CD44, measured using immunofluorescence, Western blots and FACS experiments, was unchanged. Hyal2 coimmunoprecipitated with CD44 in the presence of low and high levels of extracellular HA.

Thus, Hyal2 can decrease the efficiency of HA receptors, mostly CD44, and reduce cell coat. The impact of these events on cell signaling and behavior, in particular on the adhesiveness and invasiveness of transformed cells, is being explored.

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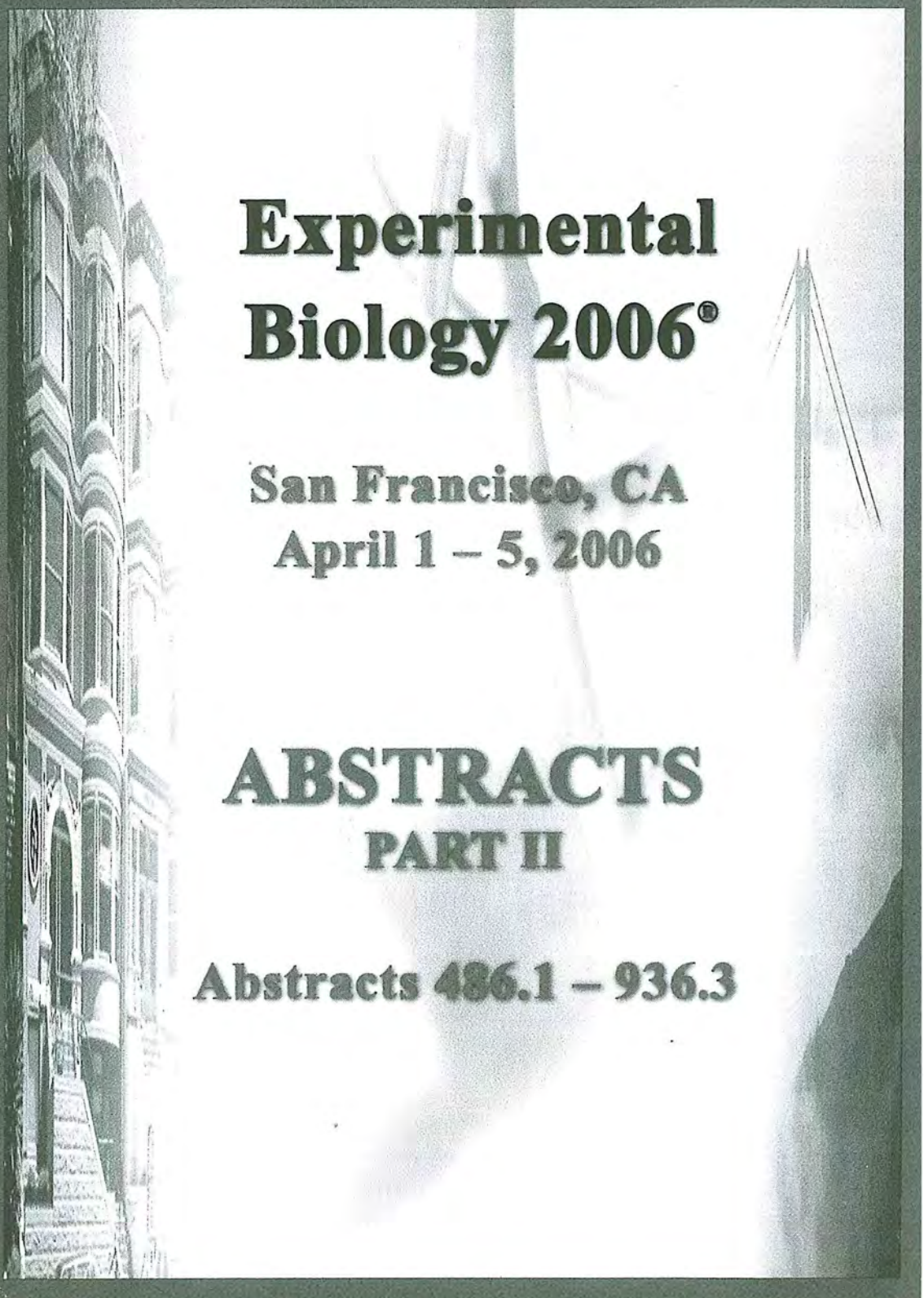

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ABSTRACTS
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