Functional Studies of Laminin-5

Abstract: Laminin-5 (Ln-5) is a basement membrane extracellular matrix macromolecule crucial to fundamental processes of epithelial morphogenesis and homeostasis. In large part Ln-5 functions rely on interactions with its two receptors, integrins alpha3beta1 and alpha6beta4, but it is not clear how such interactions translate mechanistically into cell adhesion, migration and hemidesmosome formation. A key step towards unveiling these molecular mechanisms is the definition of integrin binding sites on Ln-5. To illustrate this point, our recent uncovering of two binding sites for integrin alpha3beta1 on the LG3 and LG4 domains of the Ln-5 alpha3 chain points to novel molecular mechanisms, at the ligand level, for modulating alpha3beta1 affinity/avidity. In this proposal, we will test the hypothesis that the core of these mechanisms is proteolysis between LG3 and LG4, resulting in downregulation of cell migration. Another important focus of our proposed studies is the Ln-5 binding site for alpha6beta4, which is still uncharacterized. Disruption of alpha6beta4 /Ln- 5 binding, e.g., in inherited blistering diseases, totally compromises epithelial integrity, since this interaction is uniquely responsible for hemidesmosome assembly. We intend to define the alpha6beta4 LG binding site and test the hypothesis that it is involved in directing redistribution of alpha3beta1 and alpha6beta4 integrins as epithelial cells switch from migration to hemidesmosome based static adhesion. Our proposal is divided into 3 Aims. In Aim 1, we will carry out a comprehensive analysis of alpha3beta1 and alpha6beta4 binding sites on Ln-5 by producing LG recombinant domains and Ln-5 chimeric molecules, and testing them in adhesion, migration, hemidesmosome formation and direct binding assays: In Aim 2 we will test the hypothesis that loss of LG4 initiates a switch from migration to static adhesion by i) measuring the Kd of a3(1 interactions with LG3 and LG4 and testing if they establish an affinity/avidity threshold for migration; ii) determining whether LG4 removal halts migration and starts recruitment of hemidesmosomal components; iii) testing whether specific LG domains direct the trafficking of alpha3beta1 and alpha6beta4 between podosomes and hemidesmosomes. In Aim 3 we will study the biological consequences of integrin interactions with specific LG domains in two Ln-5 dependent organotypic systems, formation of mammary gland acini and of kidney tubes. At a basic research level, our results will enable us to translate molecular scale quantitation of integrin/Ln-5 binding into cellular scale mechanisms of adhesion and migration, and then observe how these mechanisms operate at the scale of epithelial morphogenesis. Our findings should be relevant to human health as they will enhance our ability to manipulate physiological and pathological processes like wound healing, tissue regeneration and cancer invasion.