Basement Membrane Zone

Development of Animal Models to Study Basement Membranes

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While inherited and acquired diseases have proven invaluable in increasing our understanding of the basement membrane zone (BMZ), the identification of the genes of various BMZ proteins and the development of advanced molecular techniques has allowed for the development of many new animal models, which present a great opportunity for increasing our knowledge of BMZ biology, the pathogenesis of disease and ultimately the treatment of these diseases.

Three types of animal models have significantly advanced our understanding of inherited diseases of the BMZ and now provide *in vivo* paradigms to further study their pathogenesis and to test potential therapies. These animal models include transgenic mice, immunocompromised mice with transplanted human skin equivalents and spontaneously occurring basement membrane zone (BM) disease in large animals. Distinct transgenic mouse models demonstrate many of the features of inherited BM diseases, including the simplex, junctional, hemidesmosomal and dystrophic forms of epidermolysis bullosa (EB). This was first established by the Fuchs laboratory (Vassar *et al.*, 1991), who showed that transgenic mice expressing a mutated form of keratin 14 demonstrated basal cell cytolysis and intraepithelial blistering that mimicked EB simplex to a remarkable degree. This landmark study was the first to demonstrate that a keratin gene mutation was linked to an inherited disease in humans. Since that time, other transgenic models have targeted a variety of BM components to establish transgenic models of hemidesmosomal (integrin-α6, integrin-β4 and plectin), junctional (laminin 5 subunits) and dystrophic (type VII collagen) forms of EB.

Unfortunately, these transgenic animals result in neonatal lethality that has precluded long-term studies to test therapeutic strategies targeted at phenotypic reversion of the BM defect. To overcome this obstacle and to establish humanized animal models of inherited BM disorders, human skin equivalents have been fabricated from primary cells derived from EB patients and then grafted to immunocompromised mice. This novel approach was first used by the Khavari laboratory (Ortiz-Urda *et al.*, 2003) to study the phenotypic reversion of a skin equivalent generated *in vitro* using keratinocytes from a recessive dystrophic EB patient and then transplanted to scid/scid mice. These grafts were injected with an intradermal bolus of recessive dystrophic EB fibroblasts that were engineered to correct their type VII collagen deficiency through overexpression of this protein. Strikingly, this exogenous type VII collagen was delivered to the BMZ and resulted in the prevention of sub-epidermal blistering. Subsequently, a similar animal model has been adapted by the Woodley laboratory (Woodley *et al.*, 2004) to correct a recessive dystrophic EB phenotype by direct injection of a recombinant form of human type VII collagen. The successful reversion of this disease phenotype supports future use of protein-based therapy to treat inherited skin disorders of BM.

Finally, spontaneously occurring, large animal models of inherited BM disease have been well characterized and are now available to study these diseases in an immunocompetent background. These naturally occurring diseases mimic the simplex (bovine), hemidesmosomal (dog), junctional (horse, dog) and dystrophic (sheep, dog, cat) forms of EB and have been linked to the characteristic genotypes and phenotypes seen in their human counterparts (Spirito *et al.*, 2002; Capt *et al.*, 2005; Hengge, 2005). Interestingly, phenotypes observed in these animals demonstrate varying degrees of severity that have been linked to levels of functional BM proteins. For example, a breed of German Pointer manifests an intronic mutation in the gene encoding the α3 chain of laminin 5 that interferes with RNA maturation, decreases levels of functional laminin 5 and results in a mild phenotype similar to that seen in non-Herlitz junctional EB. In contrast, a horse model that mimics severe Herlitz junctional EB has been linked to a base pair insertion generating a premature termination codon that leads to complete loss of laminin-α2 expression. These large animal models provide new opportunities to test delivery of therapeutic genes or gene products to correct molecular defects underlying these

conditions, while testing host immunotolerance. All of these animal models now play a critical role in validating discoveries made *in vitro,* in an *in vivo* context, to help close the loop between basic scientific investigation of inherited BM disease and translational discovery paradigms that will lead to the amelioration of these disease conditions (Jiang and Uitto, 2005).

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