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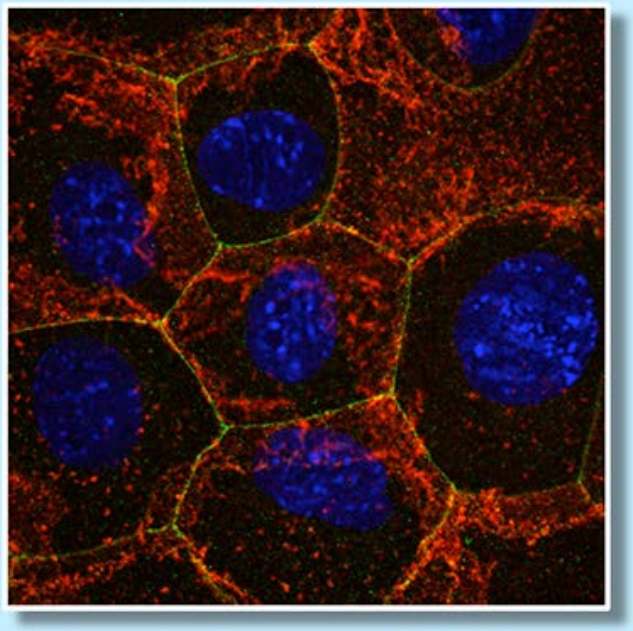

Nectin 2 (green) localizes to boundaries between wild-type keratinocytes *in vitro* (red: α-catenin, blue: nuclei)

Development and Production of shRNA Lentiviruses Targeting Nectin-2 and -4

Nectin-1, -2, -3, and -4 are transmembrane molecules whose extracellular domains form intercellular adhesions in a manner dependent on their cytoplasmic binding partner, afadin. Some nectin mutations in humans have been linked to pathologies that strongly resemble those seen in afadin mutants, such as cleft palate and ectodermal dysplasia. Thus, we hypothesize that afadin-related pathologies result from perturbed nectin function. However, knockout mouse models of single nectins fail to recapitulate this phenotype and viable compound knockout models do not exist. To circumvent these limitations, we used short hairpin RNA (shRNA) lentiviral vectors to target the two most understudied nectins, Nectin-2 and -4. To create these reagents, DNA oligonucleotide templates for shRNAs targeting specific transcripts were inserted into cloning vectors. Lentiviral packaging cells were co-transfected with these vectors and lentiviral assembly plasmids to produce virus. Eight shRNA lentiviruses - four per target nectin - have been generated. Quantitative PCR was used to determine knockdown efficiency of these viruses *in vitro*. Promising candidates were identified for continued development, although additional testing is needed to confirm their efficacy. These viruses will be used to generate stable knockdown cell lines and adapted for *in vivo* applications. They can be used in combination to target multiple nectins simultaneously, making them valuable molecular tools for future investigations on the role of nectins in development and disease.