Controlling hemidesmosome dynamics by phosphorylation of the integrin β4 subunit

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Summary

Hemidesmosomes (HDs) are protein complexes that are important for maintaining the integrity of epithelial tissues. HDs mediate stable adhesion of the basal epithelial cells to the underlying basement membrane, by linking the keratin filament system to the extracellular matrix. This link consists of the integrin α6β4, that binds to Ln-332 in the basement membrane, and plectin, that binds to the keratin filament system.

The stable adhesion of the basal keratinocytes to the underlying basement membrane is not desired when cells have to migrate and proliferate during wound healing. In order to allow migration of keratinocytes, HDs have to be disassembled. The production of cytokines and growth factors, such as EGF, during wound healing, have been shown to be important in the regulation of HD disassembly. In this study we investigated the mechanism of growth-factor-induced HD (dis)assembly. An overview of the background of HD (dis)assembly and the signal pathways involved in the phosphorylation of residues in normal keratinocytes and in carcinoma cells is given in chapter 1. EGFR activation can induce HD disassembly by phosphorylation of serine/threonine residues on the intracellular domain of the integrin β4 subunit. Phosphorylation of tyrosine residues seems to be restricted to carcinoma cells, as these phosphorylation events stimulate the growth factor signaling pathways activated during tumor cell proliferation and invasion.

The interaction between the integrin α6β4 and plectin is essential for HD assembly and is mediated by two sites of interaction. The primary site of interaction involves the first and second FNIII repeat and part of the CS of β4 and the plectin ABD, whereas the secondary site of interaction involves the C-tail of β4 and the plectin plakin domain.

In chapter 2, we studied the regulation of the primary site of interaction between β4 and the plectin ABD by serine phosphorylation of the integrin β4 subunit downstream activation of the EGFR. In contrast to previous studies, we have shown that stimulation with EGF or PMA did not induce the phosphorylation of the integrin β4 subunit on S1360, but on S1356 and S1364 instead. Moreover, we found that ERK1/2 and p90RSK1/2 phosphorylate β4 on S1356 and S1364 respectively. Phosphorylation of β4 on both serine residues prevented the primary interaction between β4 and the plectin ABD. Moreover, this induced the disassembly of both type I and II HDs by affecting the dynamic behavior of β4 and this led to a decreased strength of keratinocyte adhesion to Ln-332. Furthermore, we
showed that phosphorylation of β4 on S1356 is implicated in mitosis, when cell rounding reduces the overall cell-substrate area and the number of HDs. The role of the secondary site of interaction between β4 and the plectin plakin domain in HD (dis)assembly is further addressed in chapter 3. We have shown that the C-tail is in close proximity to the CS of β4, thereby forming a scaffold binding platform for the plectin plakin domain. Mimicking the phosphorylation of β4 on the conserved T1736 disrupted the interaction between the C-tail of β4 and the plectin plakin domain and contributed to the disassembly of HDs in keratinocytes. Furthermore, we showed that β4 is phosphorylated on T1736 by PKD1 and that this required not only the activation of PKD1, but also its translocation to the plasma membrane where β4 is primarily located after stimulation with PMA.

Both the EGF-induced phosphorylation of β4 on S1356 and S1364 and the phosphorylation of β4 on T1736 by PKD1 are important for a more complete HD disassembly in keratinocytes. Nevertheless, the regulation of HD disassembly is a complex and dynamically regulated process involving many signaling pathways. The regulation and mechanism of the phosphorylation induced HD disassembly in normal keratinocytes and carcinoma cells are further discussed in chapter 4.