Plakin interactions in the hemidesmosome
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Summary

The skin forms a selective barrier that protects humans and other organisms against influences from the external environment. The epidermis constitutes the outer layer of the skin and is composed of epithelial cells (keratinocytes). The basal side of the epidermis is anchored to the underlying basement membrane. Anchorage is mainly provided by specialized adhesion structures called hemidesmosomes that are present at the cell surface of keratinocytes. Hemidesmosomes provide the linkage between laminin-5 in the basement membrane and the keratin intermediate filament system inside the cell, and thereby ascertain a strong attachment of the epidermis to the rest of the body.

The integrin α6β4 is an important component of hemidesmosomes. It is a heterodimeric transmembrane protein of which the cytoplasmic domain of the β subunit is extremely long (1000 amino acids) as compared to other β subunits (50 amino acids). The cytoplasmic domain has little characteristics in common with other proteins, except for the presence of 2 pairs of fibronectin type three repeats which are separated from each other by a connecting segment.

Two other constituents of the hemidesmosome are BP230 and plectin. These closely related cytoskeletal linker proteins belong to the same family of proteins called the plakins. In addition to these proteins, also BP180 and the tetraspanin CD151 are present in hemidesmosomes.

The studies presented in this thesis have focused on the analysis of interactions between different hemidesmosomal components, with primary interest in the involvement of plakins.

In Chapter 1 an overview of the current literature on HDs is given.

In Chapter 2 the effect of 2 mutations in β4, initially detected in patients, in tissue culture and yeast two-hybrid experiments is described. Both mutations could be mapped to the same region on the first pair of FNIII repeats after extrapolation on the 3 dimensional structure of these domains. Introduction of β4 constructs containing these mutations in yeast two-hybrid experiments, results in the abrogation of binding between the mutants and the ABD of plectin, while binding of the ABD to wild type β4 is not affected. The introduction of these mutations in full length cDNA constructs of β4, followed by their expression in cell lines, also results in the loss of plectin recruitment into hemidesmosomes by the mutants. Other mutants of β4, that have also been tested for their ability to bind plectin, did not show an effect on recruitment. These results explain why patients carrying these mutations develop a skin disease called epidermolysis bullosa, because a crucial binding between β4 and plectin is broken.

In Chapter 3 studies on the interactions between BP230 and BP180 are described. To detect these interactions we made use of the yeast two-hybrid system. With this technique we identified direct interactions between the two proteins. Next, we determined the minimal regions involved in binding, by progressively shortening the BP180 and BP230 constructs used in the assay. With the knowledge of the minimal binding surfaces required for the binding of BP180 to BP230, we determined the importance of these regions in the assembly of hemidesmosomes. For this, we have utilized a keratinocyte cell line obtained from a patient who does not express BP180. Without BP180, these cells assemble hemidesmosome-like structures containing α6β4 and plectin, but lacking BP180 and BP230. Introduction of full length BP180 into these cells results in the expression of BP180, and the assembly of hemidesmosomes, containing in addition to α6β4 and plectin, also BP180 and BP230. However, when a mutant BP180 is used, which lacks the binding surface for BP230, the formed hemidesmosome structure lacks BP230. Since we did not have a cell line derived from a patient lacking BP230 at our disposal, we generated haemaglutinin-tagged BP230, and determined whether this clone is recruited into hemidesmosomes. When a mutant was used that no longer contains the binding site for BP180, then again, BP230 is not recruited into HDs. We thus demonstrated that the recruitment of BP230 into HDs is completely dependent on BP180. In a cell line of a patient lacking β4, no hemidesmosomes are formed, neither are complexes of BP180 and BP230. Thus, even though recruitment of BP230 into hemidesmosomes is dependent on BP180, BP180 by itself is not sufficient to recruit BP230.
In addition, we have determined that BP180, next to BP230, also interacts with the plakin protein plectin and that the binding sites on plectin and BP230 are homologous. Furthermore, an interaction between BP230 and β4 was identified and we determined the minimal binding surface for β4 on BP180. Based on all the interactions identified in this chapter, we have proposed an hierarchical model for the assembly of hemidesmosomes.

In the studies described in Chapter 4, we focused on the interaction between plectin and β4, that has previously been identified in our laboratory. Initially, we determined that the actin binding domain (ABD) of plectin interacts with the first pair of FNIII repeats of β4. At that time, it was also determined that this was the only binding site for β4 on the N-terminal region of plectin. However, there is hardly any recruitment of the ABD of plectin into hemidesmosomes, when introduced in cell lines. Based on this finding, we decided to once again look into the β4 binding capacities of the N-terminus of plectin.

We started with the analysis of a cell line that stably expresses a β4 subunit truncated at amino acid 1355 (β1355) and showed that plectin was recruited into HDs in these cells. Since we already showed previously that β41328 is unable to recruit plectin, we closely examined the sequence between 1328 and 1355 and identified two proline residues that are essential for the recruitment of plectin, since when replaced by alanine in a full length β4 construct, recruitment is abrogated. Next, we identified an additional binding site for β4 in the first 606 residues of plectin (plectin1-606). This fragment is able to interact with both the second FNIII repeat, a region in the connecting segment, and the C-tail of β4. With co-immunoprecipitations we were able to show involvement of all three regions in the binding of plectin to β4.

Introduction of plectin1-606 in cells, however, did not result in the recruitment of this fragment into hemidesmosomes but rather it decorates a filamentous network. Extension of plectin to contain also the rod domain was required for recruitment into hemidesmosomes. Also in the absence of the ABD, recruitment into hemidesmosomes occurs, however only when the cells also contain a plectin ABD that can interact with the first pair of fibronectin type III repeats. The results indicate that the extra binding sites on plectin can only be used when a plectin ABD is bound to β4 and that β4 can interact with two plectin molecules at the same time.

Chapter 5 covers the identification of crucial amino acids in the ABD of plectin that are involved in binding to the first pair of FNIII repeats in β4.

We first investigated the specificity of ABD binding to β4 by testing ABDs of different proteins for their binding to β4. From this experiment it became apparent that only the ABD of plectin and that of a variant of BP230, dystonin, bound to β4, while all other ABDs tested did not. Next, we investigated the involvement of the sequences in front of the ABD in the interaction with β4. These sequences do not directly interact with β4, but do influence the binding. Careful analysis of the sequences of ABDs, has lead to the identification of a region that is present in plectin and dystonin, but not in any of the other ABDs analyzed. Mutation of two amino acids in this region into the respective residues present in another ABD (that does not interact with β4), results in the inability to bind to β4 in yeast. In co-immunoprecipitations, the introduction of these mutations greatly reduced the binding to β4. Also in cell transfection experiments, the recruitment of the mutant into hemidesmosomes was hampered. Co-sedimentation experiments showed that the introduction of these mutations did not affect the binding to actin.