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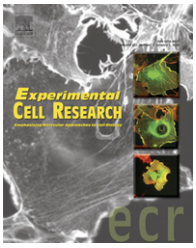

Research Article

Keratin 8 modulation of desmoplakin deposition at desmosomes in hepatocytes

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ABSTRACT

Keratins, the intermediate filament proteins of epithelial cells, connect to desmosomes, the cell–cell adhesion structures at the surface membrane. The building elements of desmosomes include desmoglein and desmocollin, which provide the actual cell adhesive properties, and desmoplakins, which anchor the keratin intermediate filaments to desmosomes. In the work reported here, we address the role of keratin 8 in modulating desmoplakin deposition at surface membrane in mouse hepatocytes. The experimental approach is based on the use of keratin 8- and keratin 18-null mouse hepatocytes as cell models. In wild-type mouse hepatocytes, desmoplakin is aligned with desmoglein and keratin 8 at the surface membrane. In keratin 8-null hepatocytes, the intermediate filament loss leads to alterations in desmoplakin distribution at the surface membrane, but not of desmoglein. Intriguingly, a significant proportion of keratin 18-null hepatocytes express keratin 8 at the surface membrane, associated with a proper desmoplakin alignment with desmoglein at desmosomes. A Triton treatment of the monolayer reveals that most of the desmoplakin present in either wild-type, keratin 8- or keratin 18-null hepatocytes is insoluble. Deletion analysis of keratin 8 further suggests that the recovery of desmoplakin alignment requires the keratin 8 rod domain. In addition, similarly to other works revealing a key role of desmoplakin phosphorylation on its interaction with intermediate filaments, we find that the phosphorylation status of the keratin 8 head domain affects desmoplakin distribution at desmosomes. Together, the data indicate that a proper alignment/deposition of desmoplakin with keratins and desmoglein in hepatocytes requires keratin 8, through a reciprocal phosphoserine-dependent process.

Introduction

Keratins (K) constitute the most diverse family of intermediate filament (IF) proteins, with at least 20 cytoplasmic proteins that are divided into type I (K9–K20) and type II (K1–K8) subclasses [1–4]. Keratin IFs are obligate heteropolymers composed of members of the two subclasses, and subsets of the two types are normally expressed in equal molar ratio in diverse epithelia [4,5]. Simple epithelial cells all contain K8/K18, the first IF proteins to be expressed during embryogenesis

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[6–10]. K8/K18 expression becomes prominent in the simple epithelium and the periderm at late embryonic stage and restricted to simple single-layered epithelia, such as liver, at the post-natal stage [8,10–12]. Like all IF proteins, K8 and K18 consist of a central α-helical (rod) domain flanked by N-terminal and C-terminal globular "head" and "tail" domains [13,14]. The rod domain exhibits highly conserved motifs at both extremities, and this domain constitutes a major driving force during the assembly of IF proteins [15,16]. The head and tail domains contribute to most of the structural heterogeneity of IF proteins [17] and are largely responsible for the spatio-temporal IF organization and interactions with various associated proteins [18]. In fact, from work performed on simple epithelial cells, there is ample evidence indicating that these interactions are often part of sequential events that can be initiated by cell growth and a variety of cell stresses, which lead to differential K8/K18 phosphorylation at the terminal domains, and to related IF re-organization [19]. However, K8/K18 exist not only as a fibrillar network, but also as a soluble tetramer pool, which proportion is also phosphorylation-dependent [18]. Nevertheless, the contribution of the fibrillar and phosphorylation status of K8/K18 to their interaction with associated proteins remains unclear.

One key function that appears to be common to all keratin IFs is their capacity to maintain surface membrane integrity largely through interactions with the intercellular adhesive junctions, the desmosomes [20,21]. Keratin IFs exhibit unique viscoelastic properties that render them much resistant to cell deformation and other mechanical stresses [17,22], and since desmosomes link keratin IFs of one cell to those of its neighbors, together keratin IFs and desmosomes form an integrated and mechanically resilient network across the epithelium [20,21]. The main building elements of desmosomes are the cadherin desmoglein (Dsg) and desmocollin, members of the armadillo family such as plakoglobin and plakophilin, and the plakins desmoplakins (DP) and plectin, two typical cytoskeletal linker proteins [20,21,23,24]. These multifunctional proteins are assembled according to a hierarchy of protein–protein interactions, with DP being largely responsible for anchoring the keratin IFs to the desmosomes [25,26]. Although an ectopically expressed DP tail domain co-aligns with and disrupts keratin IF networks in non-polarized cells of simple epithelial origin [27], *in vitro* binding assays have failed to demonstrate a direct interaction of this DP domain with K8, despite the fact that the same DP domain binds to K1 and K5 [28]. In fact, comparative two-hybrid analyses of the ability of the DP tail domain to bind keratins have revealed that, unlike K1 and K5, which can attach directly to this DP domain, in the absence of their type I partner, K8 apparently requires the presence of K18 to interact with DP [29,30]. Moreover, in line with the fact that protein–protein interactions can be modulated by changes in Ser phosphorylation status [18], there is convincing evidence that phosphorylation of Ser2849 of DP can negatively regulate its interaction with K8/K18 IFs [29–31].

But conversely, the evidence for the involvement of keratin phosphorylation in this K8/K18–DP interaction is lacking [18,19]. Three phosphoSer residues, i.e. Ser24, Ser74 in the head domain and Ser432 in the tail domain, have been identified on human K8 [32,33] and two phosphoSer sites, Ser34 and Ser53, have been documented in the head domain of K18 [34]. Perhaps, the most relevant information on phospho-Ser involvement in protein–protein binding comes from work showing that, upon a selective phosphorylation of Ser34, K18 binds to the scaffolding 14-3-3 ζ protein and that a mutation at Ser34 perturbs the 14-3-3 protein distribution [34,35]; from a functional standpoint, this mutation disturbs G2/M phase transition and causes aberrant cytokinesis [36]. In addition, Raf-1 kinase is known to bind to K8 and is released upon Raf-1 hyperphosphorylation in response to stress; in turn, K18 via its Ser53 and Ser34 residues is a physiological substrate for the released Raf-1 [37]. However, essentially nothing is known about the downstream partners in spite of several observations indicating that K18 Ser53 and K8 Ser432 are phosphorylated during mitosis and that K8 Ser74 is phosphorylated at mitosis, in response to a variety of cell stresses, and during apoptosis [33]. The phosphorylation motif at Ser24 is conserved among type II keratins, and since keratin IF binding to desmosomes is common to all epithelial cells [32], our hypothesis is that this particular phosphoSer site on K8 may modulate K8/K18–DP interaction.

Hepatocytes exhibit typical epithelial cell polarity features and their IFs consist solely of the K8/K18 pair. Since the loss of one keratin normally leads to the degradation of its partner [38–40], hepatocytes provide a unique cell model to address the functions of these simple epithelium keratins [36,41–46]. By using a K8-null mouse model [47], where the lack of K8 leads to the absence of K18, we have shown previously that K8/K18 IFs contribute to the maintenance of the integrity of the hepatocyte surface membrane in response to mechanical stress [42]. K18-null mice have also been generated and, surprisingly, K8 was found to accumulate as aggregates in hepatocytes at old age [43], but the significance of this K8 persistence is unclear. In the work reported here, we investigate the role of K8/K18 in regulating DP deposition at desmosomes in mouse hepatocytes in primary culture. Comparative analyses on K8-null and wild-type (WT) mouse hepatocytes reveal the presence of Dsg-labeled desmosomes in the absence of K8/K18 IFs, but the loss of K8/K18 perturbs the deposition of DP. Intriguingly, K18-null hepatocytes express some K8 at the surface membrane, associated with a proper DP alignment with Dsg and K8 at desmosomes. Moreover, K8-deletant cDNA transfer in K8-null hepatocytes supports the hypothesis of a preferential interaction of DP with the rod domain of the keratin. In addition, the re-insertion of a K8 phosphoSer24, a phosphoSer74 or a Ser432 mutant suggests that a change in Ser24 phosphorylation modulates the interaction in a selective manner.

Materials and methods

Reagents

Isoflurane was purchased from Abbott Laboratories Ltd (Montreal, Canada). EHS Matrigel Brand (#354234) was bought from BD Pharmingen (Mississauga, Canada). All other reagents were purchased from Sigma Chemical Co. (Mississauga, Canada). Human K8 cDNA was obtained from ATCC (Rockville USA). The antibodies used here were the following: rat anti-