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Review

Cell biology of the intercalated cell in the kidney

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ABSTRACT

The intercalated cell of the collecting tubule of the mammalian kidney is specialized for the transport of H^+ and HCO_3. They exist in two forms; one specialized for acid secretion and the other secretes HCO_3 into the urine. We discovered many years ago that feeding animals an acid diet converts the HCO_3 secreting form to an acid secreting type. Here I discuss the molecular basis of this transformation. The conversion of the cell types is mediated by an extracellular matrix protein hensin (also known as DMBT1). However much remains to be identified in the differentiation of these cells.

1. Introduction

The earliest multicellular organisms arose by differentiation of unicellular types to form structures that allowed new functions for these animals. Of these functions, two were obviously necessary, germ cells for self-perpetuation and epithelial cells that allow separation of an internal milieu from the surrounding waters. Indeed, the earliest such organisms, sponges contain only these two cell types. In vertebrates the number of types of epithelial cells far out number other types of cells, existing in essentially most organs. Most of these epithelia organs are composed of different segments or tissues each with its own specific epithelial type. For instance in the pancreas, the epithelial cells of the acini and the ducts are quite different but the acinus or the duct each has only one characteristic epithelial cell type. In some epithelia, however there are two or more cell types that exist in a mosaic pattern in the same tissue. This so called salt and pepper pattern exists in the skin and gills of fishes [1], in the amphibian and reptilian skin [2], the inner ear [3] and several distal epithelia in the kidney including the urinary bladder of amphibia and reptiles and the mammalian the kidney collecting tubule [4]. In addition during early mouse embryo development the primitive endoderm [5] has a stage in which cells destined to have different fates exist together in a seemingly random manner.

2. Intercalated cells and H^+/HCO_3 transport

The function of the intercalated cells has been intensively studied in renal epithelia. In the turtle urinary bladder, Steinmetz [6] demonstrated that the epithelium secretes H^+ and since it became known that this epithelium contains "mitochondria-rich" cells which were enriched in carbonic anhydrase [7], an enzyme critical for acid secretion it was possible to ascribe this function to these cells. Later it was discovered that this epithelium was also capable of secretion of HCO_3 in exchange for Cl [8]. Interestingly, Steinmetz also identified two morphologic varieties of these mitochondria-rich cells that they termed α and β [9]. Similar studies to those in turtle bladder also identified intercalated (or mitochondria-rich) cells in the collecting tubules as well as both acid secretion and HCO_3 secretion.

Especially in cortical collecting tubules isolated from rabbit kidneys the dominant form of transport was HCO_3 secretion. Because we had discovered that the acid secreting (α) intercalated cells had vigorous apical endocytosis [10] we perfused rabbit cortical collecting tubules with an endocytosis marker and found that there remained a large fraction of intercalated cells in these HCO3-secreting tubules that had no apical endocytosis. We then concluded that these must be the HCO_3-secreting β type [11]. An apical $Cl:HCO_3$ exchanger and a basolateral H^+ ATPase mediate secretion of base by the β cells while α cells secrete acid by an apical H^+ ATPase and a basolateral $Cl:HCO_3$ exchanger. In both cell types, it is same vacuolar ATPase that is located in the apical membrane of the α form and in the basolateral membrane of the β type[12]. However, there is now general agreement that the apical $Cl:HCO_3$ exchanger of the β form is pendrin while the basolateral exchanger

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of the α cell is an alternately spliced form of the red cell anion exchanger, AE1. Remarkably, we discovered that metabolic acidosis converts the collecting tubule from a state of HCO_3 secretion to HCO_3 absorption (i.e., H^+ secretion). We found that this was due to an increase in the α type with a quantitatively equal decrease in the β form, a situation that we interpreted to mean that the alkali secreting cell converted to an acid secreting variety. The reversal of the polarized distribution of the H^+ ATPase attracted much attention among cell biologists since it contradicted the deterministic idea (current in the mid 1980's) that polarized sorting of proteins in epithelia was due to the presence of targeting sequences in the proteins and hence was "immutable". We initially proposed and had experimental evidence for the reversal of targeting of the AE1 protein as well, but recent studies by many others showed that the apical Cl:HCO_3 exchanger is pendrin (Slc26A4) [13], hence we have to modify our initial hypothesis by saying that acidosis reverses the polarized distribution of only the H^+ ATPase, removes pendrin from the apical membrane and induces the AE1. We have provided compelling evidence for the existence of this plasticity using a variety of in vitro and in vivo methods. Over the past ten years of study of this plasticity, we have come to the surprising conclusion that the conversion of the β to the α-intercalated cell is a manifestation of epithelial terminal differentiation with the α type being the more differentiated form of the cell. Hence the study of the molecular basis of this plasticity will shed light on the process of epithelial differentiation, a process that at the center of organogenesis and of diseases such as cancer.

To identify the molecules that mediate this conversion we purified β-intercalated cells from rabbits using peanut lectin as a marker. We then transfected these cells with a temperature -sensitive SV-40 large T antigen to generate an immortalized clonal intercalated cell clonal line [14]. We found that these cells did not respond to an acid stimulus but retained the ability to convert from a β phenotype to α when the seeding density of the β cells was increased by 100-fold. When we seeded them at sub-confluent density they became β-intercalated cells at confluence. But when seeded at super-confluent density, this clonal cell line became identical to α-intercalated cells [15]. This was not simply a matter of density, since cells seeded at low-density at confluence had essentially the same cell number, yet they remained β-intercalated cells. To identify the mechanism of the effect of high density we first thought that there might be a factor that is being secreted in the medium and of course its concentration in high density cells would be greater. But detailed efforts at concentrating conditioned media led nowhere. We then tested the idea that perhaps at high density the cells deposit an extracellular matrix protein on the filter that can instruct the cells to change a phenotype. We seeded cells at high density on filters, then solubilized and discarded the cells. On these "conditioned filters" we seeded cells at low density. Remarkably the cells acquired the phenotype of high density. Hence, high-density cells deposited an extracellular matrix protein that instructed low density to become α−intercalated cells. These results conclusively demonstrate that it was not density *per se* that produced the change in phenotype rather density at seeding. We purified this protein and termed it hensin and found that it is expressed in most epithelia. In particular we found that when β-intercalated cells were exposed to an acid medium it lost its apical Cl:HCO3 exchanger while developing basolateral Cl:HOC3 exchange and that blocking antibodies to hensin prevented this process [16].

3. Hensin deposition in the ECM requires polymerization

Hensin is expressed as a monomer in the β IC that is secreted largely into the basolateral medium. However, in the media of α-intercalated cells many multimers of hensin are present and the majority is deposited in the extracellular matrix in an "insoluble" form with an apparent molecular mass in excess of 10 million. When hensin was purified from the ECM a fibrillar structure appeared on negative staining electron microscopy [17]. Only the ECM from of hensin is capable of inducing the conversion of β-intercalated cell to α-intercalated. The earliest step causing hensin deposition is binding of monomeric hensin to an activated αvβ1 integrins. Blocking antibodies to β1 integrin prevented hensin polymerization and activating antibodies were able to polymerize hensin and induce conversion of the phenotypes [18]. Two other proteins need to be secreted to complete the polymerization; galectin 3 must bind to hensin in the ECM [19,20]. We found that another protein cyclophilin A, is also needed. Its enzymatic activity (*cis*/*trans* prolyl isomerase) is what is needed since inhibition of this enzyme prevented hensin polymerization. Hensin contains several proline rich regions which under the influence of cyclophilin A might be converted into an all-*trans* form which would be predicted to produce a super-helix [21]. Interestingly these proteins, galectin 3 and cyclophilin A do not contain signal sequences and hence must be secreted by the non-classical secretory pathway.

4. Conversion of β to α intercalated cells is similar to terminal differentiation

While the two types of intercalated cells have opposing H^+ transport properties they differed in many other ways both in vitro and in vivo. High-density cells (α-intercalated cell) were twice as tall as low-density cells. The apical surface of α-intercalated cells has exuberant apical microvilli and vigorous apical endocytosis. β-intercalated cells had hardly any apical microvilli and essentially no apical endocytosis [22,23]. Apical microvilli require the presence of elaborate cytoskeleton and indeed we found that high-density cells induce the production of villin and cytokeratin19, proteins that are absent in low-density cells. All of these phenomena were induced by purified hensin. All the characteristics of high density intercalated cells are those of terminally differentiated columnar epithelia and low density intercalated cells remind one of early embryonic epithelia (e.g., primitive endoderm, kidney tubules at E 12, early small intestine) where the cells are flat have no apical microvilli, no apical endocytosis no exocytosis. Indeed, when we cultured embryonic stem cells on hensin, we found that they developed into columnar epithelia with apical microvilli and apical endocytosis [24]. When we performed a global knockout of hensin we found that the embryos died at E 4.5, a time when the first columnar epithelium, the visceral endoderm developed. Terminal differentiation in some epithelia continues in adult life e.g., in the intestine, the crypt cells are less differentiated than the villus cells and hensin is localized in the ECM only underneath the absorptive cells of the villus in the intestine; similarly hensin is located in the ECM of luminal cells of the prostate but not in that of the less differentiated basal cells. Many cancer biologists believe that blockade of terminal differentiation is a critical determinant of oncogenesis and the human orthologue of hensin (DMBT1), was found to be deleted in a vast number of epithelial cancers [25]. Hence we suggest that hensin might be involved in the terminal differentiation of other epithelia. I use the term terminal differentiation in the modern sense in that it is an advanced state of specialization rather as a state where proliferation no longer is possible. Indeed, studies have shown that acidosis can induce proliferation of the α-intercalated cells [26,27].