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Isoform-specific up-regulation of plasma membrane Ca^{2+}ATPase expression during colon and gastric cancer cell differentiation

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Abstract

In this work we demonstrate a differentiation-induced up-regulation of the expression of plasma membrane Ca^{2+}ATPase (PMCA) isoforms being present in various gastric/colon cancer cell types. We found PMCA1b as the major isoform in non-differentiated cancer cell lines, whereas the expression level of PMCA4b was significantly lower. Cell differentiation initiated with short chain fatty acids (SCFAs) and trichostatin A, or spontaneous differentiation of post-confluent cell cultures resulted in a marked induction of PMCA4b expression, while only moderately increased PMCA1b levels. Up-regulation of PMCA4b expression was demonstrated both at the protein and mRNA levels, and closely correlated with the induction of established differentiation markers. In contrast, the expression level of the Na^+/K^+–ATPase or that of the sarco/endoplasmic reticulum Ca^{2+}ATPase 2 protein did not change significantly under these conditions. In membrane vesicles obtained from SCFA-treated gastric/colon cancer cells a marked increase in the PMCA-dependent Ca^{2+} transport activity was observed, indicating a general increase of PMCA function during the differentiation of these cancer cells.

Because various PMCA isoforms display distinct functional characteristics, we suggest that up-regulated PMCA expression, together with a major switch in PMCA isoform pattern may significantly contribute to the differentiation of gastric/colon cancer cells. The analysis of PMCA expression may provide a new diagnostic tool for monitoring the tumor phenotype.

Keywords: Gastric/colon carcinoma; Ca^{2+} homeostasis; Differentiation induction; Plasma membrane Ca^{2+}ATPase; Gene expression

Abbreviations: PMCA, plasma membrane Ca^{2+}ATPase; SERCA, sarco/endoplasmic reticulum Ca^{2+}ATPase; ER, endoplasmic reticulum; 5F10, pan-anti-PMCA monoclonal antibody; JA9, anti-PMCA4 monoclonal antibody; JA3, anti-PMCA4b monoclonal antibody; PL/IM 430, pan-anti-SERCA3 monoclonal antibody; IID8, pan-anti-SERCA2 monoclonal antibody; CEA, carcinoembryonic antigen; DPP-IV, dipeptidyl-peptidase IV; SCFA, short chain fatty acid; HDAC, histone deacetylase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; BSA, bovine serum albumin; RT, reverse transcriptase; CP, crossing point; TCA, trichloroacetic acid; PMSF, phenylmethyl-sulfonyl-fluoride; TES-TEA, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid-triethanolamine; PVDF, polyvinylidene fluoride; ECL®, enhanced chemiluminescence

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1. Introduction

Plasma membrane Ca^{2+} ATPases (PMCAs) play an important role in the regulation of cellular Ca^{2+} signaling and the control of cell activation (for review see [1]). Binding of various ligands to their receptors in the plasma membrane leads to the intracellular production of D-myo-inositol-1,4,5-tris-phosphate (IP_3) and consequent release of Ca^{2+} from the endoplasmic reticulum (ER). Ca^{2+} release from the ER and ensuing Ca^{2+} influx from the extracellular space through store-operated Ca^{2+} channels lead to increased cytosolic Ca^{2+} concentration and to the activation of various Ca^{2+}- and/or Ca^{2+}–calmodulin-dependent enzymes [2,3]. As PMCAs transport Ca^{2+} ions from the cytosol into the extracellular space, by decreasing cytosolic free Ca^{2+} level these enzymes are essential for the control of the magnitude of Ca^{2+} transients, and for the termination of a Ca^{2+}-dependent cell activation event. PMCA activity also determines the cytosolic Ca^{2+} concentration of a cell at the resting state. As PMCAs are themselves activated by the Ca^{2+}–calmodulin complex, PMCA activity is involved in the control of cellular Ca^{2+} oscillations, as well [4,5].

Mammalian PMCAs are encoded by four genes (PMCA1-4 or *ATP2B1-4*), and tissue- and development-specific alternative splicing of the primary transcripts of these genes generates a multitude of PMCA isoforms. PMCA1 and PMCA4 are expressed in virtually all tissues, whereas PMCA2 and PMCA3 are primarily found in some specialized tissue/cell types [6–8]. Okunade et al. [9] studied the relative importance of PMCA1 and PMCA4. They developed and analyzed mice carrying null mutations in these PMCA genes and proposed the major housekeeping role for PMCA1, but not for PMCA4. Thus, it appears that the PMCA1 isoform fulfills housekeeping roles essential for cellular Ca^{2+} signaling and/or homeostasis, whereas other PMCA isoforms serve specialized, tissue- or cell type-specific functions.

The PMCA variants identified so far in normal gastric and colon tissues are encoded by the PMCA1 and PMCA4 genes. The presence of PMCA2 and PMCA3 gene products has not been detected in these tissue types, even by the most sensitive techniques (for details see [8]). PMCA4 codes for the full-length PMCA4b isoform and the C-terminally truncated PMCA4a splice variant. Biochemically, PMCA4b is characterized by: (i) low basal activity in the absence of calmodulin; (ii) slow activation by the Ca^{2+}–calmodulin complex; and (iii) slow inactivation of the calmodulin-activated pump [10–12]. For PMCA1 biochemical data are limited due to the difficulties encountered during the cloning and expression of its cDNA [13,14]. The affinity of PMCA1 for calmodulin appeared to be similar to that of PMCA4, but it has higher affinity for ATP and a higher susceptibility to degradation by calpain [15]. Further studies are required to understand why cells co-express PMCA1 and PMCA4, and how these pumps contribute to the cellular Ca^{2+} signaling and homeostasis.

The intestinal/colonic epithelium is in a constant state of renewal. Cells proliferate and become differentiated as they migrate from the base of the crypts towards the surface. Alterations of the tightly regulated balance between the highly proliferative/less differentiated and the non-proliferative/highly differentiated states may lead to hyperplasia, benign (polyps) or malignant tumors. The pivotal role of Ca^{2+} in the pathophysiology of intestinal/colonic epithelium is well documented (for review see [16]). Increasing cytosolic free Ca^{2+} concentration has been observed during the ontogeny of intestinal epithelium [17], moreover, the differentiation-inducing effect of increased cytosolic Ca^{2+} levels has also been published [18]. In addition, the regulation of colonic epithelial cell proliferation and differentiation by the extracellular Ca^{2+} concentration predicts a chemopreventive action of Ca^{2+} on colon tumorigenesis [18–20]. Whereas data in the literature suggest a cross-talk between Ca^{2+} homeostasis and the control of epithelial differentiation, the exact mechanisms of action of Ca^{2+} and proteins involved in Ca^{2+} homeostasis in colon carcinogenesis remain to be clarified.

Formerly, we observed that the Ca^{2+} homeostasis of the ER is remodeled during the differentiation of gastric and colon carcinoma cells, and showed that, although normal colonic epithelium expresses the sarco/endoplasmic reticulum Ca^{2+} ATPase 3 (SERCA3) proteins abundantly, the expression of these enzymes is strongly decreased in colon cancer cells. SERCA3 expression could be induced by short chain fatty acids (SCFAs) that are physiological differentiation inducers present in the gut lumen due to fermentation of dietary fibers by the colonic flora. In addition to the modulated SERCA expression pattern detected during the drug-induced differentiation of various gastric/colon adenocarcinoma cell lines, enhanced SERCA3 expression accompanied also the spontaneous differentiation of several colon cancer cell lines in post-confluent cultures without any drug treatment [21,22].

Gene expression can be controlled through deacetylation of histones. A class of agents, the histone deacetylase (HDAC) inhibitors inhibit that process and are accepted to be potent differentiation inducers [23]. Treatment of tumor cells with HDAC inhibitors therefore can contribute to the re-expression of suppressed genes and can accelerate cell differentiation [24], and these agents are promising drugs that are currently in early phase of clinical trials [25,26]. In our present study, we used SCFA-type, as well as structurally unrelated HDAC inhibitors to induce cancer cell differentiation.

As the biochemical activity, as well as the transcriptional regulation of various proteins involved in cellular Ca^{2+} homeostasis (pumps, channels and Ca^{2+} binding proteins) are modulated by Ca^{2+}, these proteins function in a tightly interconnected manner. To further characterize this homeostatic matrix, in the present work we investigated the expression pattern and the function of plasma membrane Ca^{2+} ATPases in various gastric and colon cancer cell lines, and studied the effect of cell differentiation on their expression and activity. Implication of PMCAs in the differentiation of various tissue/cell types has already been published [27–33]. Our