MAP17 and the double-edged sword of ROS

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ABSTRACT

Reactive oxygen species, ROS, are beneficially involved in many signaling pathways that control development and maintain cellular homeostasis. In physiological conditions, a tightly regulated redox balance protects cells from injurious ROS activity, but if the balance is altered, it promotes various pathological conditions including cancer. Understanding the duality of ROS as cytotoxic molecules and key mediators in signaling cascades may provide novel opportunities for improved cancer therapy.

MAP17 is a small 17-kDa non-glycosylated membrane protein that is overexpressed in many tumors of different origins, including carcinomas. Immunohistochemical analysis of MAP17 during cancer progression demonstrates that overexpression of the protein strongly correlates with the progression of most types of tumor. Tumor cells that overexpress MAP17 show an increased tumoral phenotype associated with an increase in ROS. However, in non-tumor cells MAP17 increases ROS, resulting in senescence or apoptosis. Therefore, in tumor cells, MAP17 could be a marker for increased oxidative stress and could define new therapeutic approaches. Here, we review the role of MAP17 as a putative oncogene, as well as its role in cancer and anticancer therapy.

Keywords: MAP17, Cancer, Oncogene, Reactive Oxygen Species, Tumorigenesis.

1. An Introduction to ROS

ROS may promote either proliferation or cell death, depending on the intensity and location of the oxidative burst and the activity of the antioxidant system [1, 2]. Considering the proliferation signals delivered by ROS to cancer cells, and the consequent resistance of cancer cells to pro-apoptotic signals, ROS-induced tumor cell death is likely to be induced by ROS-generating antineoplastic therapies that increase the constitutive oxidative stress above the critical threshold required for cell death.

Cancer cells develop an enhanced constitutive oxidative stress that sustains tumor growth and protects these cells against proapoptotic signals, thus promoting tumor progression [1, 3]. In experimental models, it has been shown that ROS generation in tumors and the subsequent oxidative stress actually occur at sublethal levels [4]. However, many therapeutic drugs, as well as radiotherapy and photodynamic therapy, kill cancer cells, at least in part by increasing ROS [1, 5, 6] .

Evidence exists that the role of ROS in cancer is not limited to the generally accepted genotoxic and mutagenic effects that initiate cancer. As signal transduction messengers, ROS may promote the proliferation, senescence or death of cancer cells, depending on the actual intracellular and exogenous conditions. ROS have been shown to modulate growth signals and to activate expression leading to the sustained proliferation of cancer cells [3, 7]. An emerging view is that upon oncogenic transformation, cells rapidly activate a stress response as a protective measure to overcome oncogene-induced cell death and senescence [8]. Cancer cells subject to persistent endogenous and exogenous oxidative stress were shown to develop adaptive responses primarily related to the upregulation of the antioxidant machinery. Therefore, cancer cells might become resistant to both enhanced constitutive stress and ROS-generating therapies, limiting the efficacy of the latter.

Current "ROS threshold" theories suggest that along with increases in ROS, cell responses change from proliferation to balance and then to cell death after ROS surpass a certain level [9]. Therefore, normal cells differ from tumor cells in their ability to control redox homeostasis. In normal cells, the use of antioxidants to scavenge free radicals protects from ROS-induced malignant transformation. In

tumors, antioxidant or low levels of ROS that induce antioxidant defenses appear to benefit tumor growth and could enhance anticancer therapy resistance [9]. However, redox loading of tumor cells increases ROS to close to threshold levels. Then, when both normal and tumor cells are exposed to ROS-inducing therapies, the ROS levels in tumor cells will more easily reach levels that cause cell death [6]. This theory indicates that modest increases of ROS are oncogenic, while high ROS levels suppress tumors.

Accumulating evidence implicates ROS in signaling cascades related to cell proliferation and transformation [4, 10, 11]. Ras-transformed fibroblasts overproduce ROS, and this overproduction is correlated with the activation of mitogenic signaling pathways [10]. Loss of superoxide-dismutase, MnSOD, which should elevate ROS levels, has also been correlated with a tumoral phenotype, and overexpression of MnSOD leads to the reversion of the transformed phenotype [12-15]. On the other hand, H₂O₂ is generated in response to the growth factors EGF and PDGF and is linked to growth-related signaling [11, 16]. When overexpressed in NIH 3T3 mouse fibroblasts, Nox1, an NADPH oxidase catalytic subunit, induces excessive production of ROS and a transformed phenotype with increased mitotic rates and aggressive tumor formation in athymic mice [17]. The phenotype of Nox1-transfected cells can be reversed by ROS reduction through stable expression of catalase, thereby implicating ROS as a signaling molecule [17]. Although later experiments do not support that claim [18, 19], high levels of Nox1 with increased levels of ROS have been detected in colon and prostate samples [18-22]. Nox4 was shown to sustain prostate cancer cell survival after activation by signals provided by the extracellular matrix [23]. On the other hand, decreased activity of antioxidants has been observed in ovarian cancer patients [24]. Decreased expression and activity of mitochondrial MnSOD have been reported in colorectal and pancreatic carcinomas correlated with altered redox status [25, 26]. Furthermore, various oncogenic signals such as those induced by oncogenic Raf, c-myc or Bcr-Abl were shown to be involved in increasing ROS generation via the NADPH oxidase pathway [27, 28].

The cellular targets responsible for growth and transformation affected by ROS signaling are not well known. The p42/p44 mitogen-activated protein kinase (MAPK), p38 MAPK, p70S6k, signal transducers and activators of transcription

(STAT), Akt/Protein Kinase B and phospholipase D signaling pathways are all activated by reactive oxygen species [16, 29-31]; however, in some cases, activation is indirect [32, 33]. A direct effect has been shown for protein tyrosine phosphatase-1B (PTP-1B), which is inhibited by oxidation of a thiol in the active site [34, 35] leading to increased phosphotyrosines on many cell proteins. Furthermore, ROS increase activates the PI3K pathway by direct oxidation and inactivation of PTEN and other AKT phosphatases, thus maintaining AKT activation even in the absence of a PI3K signal [36]. A variety of other targets can also be affected by ROS, including transcription factors such as NF- κ B [37], activator protein-1 (AP1) [38], PTEN [39] and p53 [40]. ROS can directly modify signaling proteins through different modifications such as nitrosylation, carbonylation, disulfide bond formation and glutathionylation [41]. Whatever the proximal target(s), ROS can reprogram the expression of enzymes and other proteins in the cell [42, 43]. DNA microarray experiments [17] indicate that up to 2% of the genes are transcriptionally regulated by ROS.

2. MAP17 is overexpressed in cancer

Functional genetic screens using retroviral delivery of high complexity cDNA libraries are valuable tools to discover new genes involved in a particular phenotypic characteristic of the tumorigenic process [44-46]. A genome-wide retroviral cDNA screen to search for genes that confer a selective advantage to cancer cells during tumorigenesis allowed us to identify MAP17 [47]. MAP17 is a small, non-glycosylated, membrane-associated 17-kDa protein that localizes to the plasma membrane and the Golgi apparatus [48]. The protein sequence contains two transmembrane regions and a hydrophobic amino-terminus of 13 amino acids encoding a PDZ-binding domain [49]. MAP17 overexpression in carcinomas was first detected by using the technique of differential display [50]. Transfection of full-length wild-type MAP17 into HT29 colon carcinoma cells decreased cell proliferation *in vitro* and tumor growth *in vivo* [51]. MAP17 binds several PDZ domain-containing proteins, including PDZK1, NHERF1, NaPi-IIa and NHE3. Overexpression of MAP17 in opossum kidney cells participates, together with PDZK1 and NHERF4, in NaPi-IIa internalization to the trans-Golgi network [52]. The physiological role of MAP17 in proximal tubules is not well known, but it does stimulate specific Na-dependent transport of mannose and glucose in *Xenopus*

oocytes [48] and human tumor cells [47]. The MAP17 gene shares regulatory elements with the stem cell leukemia gene (SCL, TAL-1), which encodes a basic Helix-Loop-Helix protein essential in the formation of the hematopoietic lineages [53, 54]. However, in non-cancerous tissues, major expression of MAP17 has only been detected in kidney cells.

In a meta-analysis of public microarray databases for different skin diseases, Noh et al. [55] discovered that MAP17 is commonly upregulated, suggesting that it may be associated with abnormal keratinocyte differentiation. MAP17 was significantly upregulated in response to interferon-gamma, interleukin 4 (IL-4), IL-6, IL-17A or IL-22 in normal human epidermal keratinocytes. Interestingly, the PDZK1 gene is localized within the atopic dermatitis-linked region on human chromosome 1q21. In an attempt to evaluate whether MAP17 regulates the expression of cornified envelope-associated genes at the 1q21 locus, such as filaggrin, loricrin and involucrin, these authors [55] found that the over-expression of MAP17 in HaCaT keratinocytes significantly decreased the expression of filaggrin, a cornified envelope-associated gene. Taken together, the Th cell cytokine-induced upregulation of MAP17 expression may be linked to the downregulation of filaggrin, which may be associated with the abnormal epidermal differentiation observed in the dermatological diseases [55].

Human MAP17 maps to chromosome 1p33, a locus commonly found to be involved in cancer; however, it is not the only interesting gene in this region. Genes coding for members of the cytochrome P450 family (CYP4B1, CYP4A11), putative oncogenes (SCL/Tal1), MCPH7, CMPK1, and members of the forkhead family (FOX E3, FOX D2) are its neighbors.

MAP17 overexpression has previously been shown to be associated with carcinomas [50, 51]. An in-depth analysis of MAP17 overexpression in carcinomas by immunohistochemistry and mRNA expression showed that the MAP17 protein is overexpressed in a large percentage of the tumors analyzed (Figure 1) and is significantly correlated with the tumor grade at least in ovarian, breast and prostate carcinomas [56]. A comparison of tumoral with non-tumoral tissues of the same patient by hybridization or by analyzing mRNA levels by Q-PCR

demonstrated an even higher percentage of tumor samples with MAP17 overexpression [56]. Overexpression was observed in more than 70% of the samples from tumors such as ovary, colon, stomach, cervix and thyroid and in approximately 50% of the samples from tumors of the lung, uterus and rectum. Although more samples need to be analyzed to confirm these high frequencies, the data suggest that MAP17 overexpression is the most common marker of tumorigenesis in carcinomas. The relevance of MAP17 as a general marker for the malignant stages of human tumors still needs to be confirmed in additional tumor types and larger cohorts. Furthermore, MAP17 expression seems to correlate with AKT473 phosphorylation and p38 T180/Y182 dephosphorylation [57].

MAP17 overexpression in carcinomas occurs mostly through mRNA amplification (Figure 2). MAP17 overexpression could be due to the ability of the MAP17 promoter to be activated by oncogenes [50, 56]. Tumorigenic progression involves progressive genetic alterations triggering oncogenic cascades [58]. In advanced stages, tumors might accumulate oncogenic alterations that result in a high probability of MAP17 promoter activation and increased transcription. This hypothesis could explain the correlation between the MAP17 overexpression and advanced tumor stages observed in many tumor types.

However, the preceding data do not provide an explanation of why MAP17 overexpression provides a selective advantage during tumorigenesis. Multiple oncogenes that activate signaling pathways directly involved in cell survival or proliferation have been discovered in previous decades. Other genes may provide an advantage to the tumoral cells, making them insensitive to physiological signals or altering their normal physiology. Although activated macrophages destroy cancer cells more effectively than normal cells, the ability to escape activated macrophages is a characteristic of tumor cells. One of the mechanisms responsible for the specific killing of tumor cells by macrophages is the production of the cytokine tumor necrosis factor alpha (TNF). Therefore, resistance to TNF may provide cancer cells with a selective advantage against host elimination. Ectopic expression of MAP17 in tumor cells prevents TNF-induced G1 arrest by impairing p21waf1 induction. However, expression of MAP17 does not inhibit TNF-induced apoptosis in Me180-sensitive tumor cells. The inhibition of TNF is

specific because MAP17 does not alter the response to other cytokines, such as IFN α . As described in the *Xenopus* oocyte system, MAP17 increases the uptake of mannose in some cells, but this effect is not responsible for TNF bypass [47].

3. MAP17 expression enhances the tumorigenic phenotype by increasing intracellular ROS

Tumor cells that overexpress MAP17 show an increased tumoral phenotype with enhanced proliferative capabilities, both in the presence and absence of contact inhibition, decreased apoptotic sensitivity and increased migration [59]. MAP17-expressing clones also grow better in nude mice. The increased malignant cell behavior induced by MAP17 is associated with an increase in ROS production, and the treatment of MAP17-expressing cells with antioxidants results in a reduction in the tumorigenic properties of these cells. The MAP17-dependent increase in ROS and tumorigenesis is dependent on its PDZ-binding domain because disruption of this sequence by point mutations abolishes the ability of MAP17 to enhance ROS production and tumorigenesis [57, 59].

MAP17 also decreases the c-Myc-induced caspase-3-like activity in Rat1 fibroblasts under low serum conditions. This decrease is in keeping with the concept of MAP17-induced PI3K/AKT signaling, in which MAP17 is able to interfere with Bax translocation to the mitochondria [36]. A fraction of PTEN protein is oxidized in MAP17-overexpressing cells. Furthermore, activation of AKT by MAP17 as measured by Thr308 phosphorylation is independent of PI3K activity. Importantly, modulation of ROS by antioxidant treatment prevented activation of AKT, thus restoring the level of apoptosis in serum starved Rat1/c-Myc fibroblasts [36]. Therefore, overexpression of MAP17 protects Rat1a fibroblasts from Myc-induced apoptosis through ROS-mediated activation of the PI3K/AKT signaling pathway [36].

The increased tumorigenic properties induced by MAP17 are associated with an increase in ROS because MAP17 increases endogenous ROS, and antioxidant treatment of MAP17-expressing cells entails a reduction in the tumorigenic properties of these cells. Two explanations can be offered for the mechanism by which ROS induce the transformed phenotype. First, reactive

oxygen generated in the presence of MAP17 may be mutagenic, causing the transformed phenotype through the induction of mutations in oncogenes or tumor suppressor genes. Alternatively, ROS generated in a MAP17-dependent manner might function as an intracellular signal, inducing a growth-related genetic program. ROS removal by antioxidant treatments decreases the malignant cell behavior induced by MAP17; thus, the second hypothesis is favored. Furthermore, in breast carcinoma cells, elimination of ectopically expressed MAP17 reduced the tumorigenic capabilities [57]. This reversibility of the phenotype indicates that the effect induced by MAP17 is largely independent of ROS-induced DNA-mutations.

However, the increased tumoral properties of carcinoma cells were not paralleled in naïve non-tumoral cells [57], indicating that MAP17 provides a selective advantage once tumorigenesis has begun. Our data demonstrate that ROS act as a second messenger that enhances tumoral properties but only in those cells where the senescence/apoptotic signal provided by ROS is uncoupled. We have found that p38 α activation at least partly mediates this response. MAP17 triggers a ROS-dependent, senescence-like response that is abolished in the absence of p38 α activation. Furthermore, in human breast tumors, MAP17 activation is correlated with lack of p38 α phosphorylation. Therefore, MAP17 is overexpressed in late-stage breast tumors, in which oncogenic activity relies on p38 insensitivity to induced intracellular ROS [57].

4. ROS increase

How are ROS increased by MAP17? One possibility is that MAP17 can increase glucose and mannose uptake, inducing an increase in metabolism with ROS as side products. However, it is also possible that a direct link with the membrane transporters ends, altering the intracellular redox balance by altering the intra/extracellular ion balance.

The structural simplicity of MAP17 and the kinetic analysis of the induced membrane transport [60] suggest that MAP17 is an activator of the capacity of endogenous uphill transporters. MAP17 could modulate the activity or the organization of membrane transporters through direct interaction as the RS1

modifier does [61] or through competition for PDZ-binding domains to alter the stoichiometry of the transporter-PDZ proteins [60].

4.1. MAP17 binding to PDZK1 (NHERF3, CAP70, NaPi-Cap1 and CLAM)

Kocher et al. [62] first described the interaction between MAP17 and PDZK1 detected by using a two-hybrid system. In a transgenic mouse model, MAP17 hepatic overexpression resulted in a liver deficiency of PDZK1, suggesting that MAP17 is an endogenous regulator of PDZK1 turnover [63]. MAP17 acts as an atypical anchoring site for PDZK1 and interacts with the NaPi-IIa/PDZK1 protein complex in renal proximal tubular cells [64].

PDZK1 belongs to the NHERF (sodium hydrogen exchange regulatory factor) family, members of which are PDZ domain-containing proteins that play important roles in regulating cell function [65]. This family contains four members, NHERF1, NHERF2, PDZK1 and IKEPP (NHERF4), that share similar homology domains [65]. NHERF1 and NHERF2 each contain 2 PDZ domains, while PDZK1 and IKEPP each contain 4 PDZ domains [66]. NHERF1 was initially identified as a membrane-associated protein that is essential for the regulation of the PKA-induced inhibition of the Na-H exchange isoform 3 (NHE3). NHERF2 was also identified as a mediator of NHE3 inhibition by cAMP. Although the different members of the family carry some overlapping functions, it is clear that there is a significant amount of specificity among them [67]. Thus, PDZK1 is a typical scaffolding protein defined by the presence of globular PDZ domains that assemble several proteins into functional complexes. PDZK1 is a critical spatio-temporal regulator of intracellular signaling in response to specific stimuli [68]. MAP17 has been shown to interact with PDZK1 and NHERF1 [69] (Figure 3A).

The NHERF proteins are primarily expressed in the polarized epithelial cells at the apical side. Kidney, small intestine and liver tissues exhibit the highest expression levels of these proteins [70, 71].

The NHERF proteins regulate cell surface expression and functional activity of transporters. Most transporters identified as binding partners belong to the ABC family [66]. In addition to transporters, other proteins have been shown to interact with NHERF proteins, including signaling proteins, hormone receptors and cytoskeleton structural elements [65, 72]. Many proteins related to the G-protein

signaling pathways were found to interact with PDZK1, and they were likely to be functionally associated with transporters.

PDZK1 forms heterooligomers with NHERF1 in vitro, which may allow the formation of an entire network of PDZ adapter proteins underneath the plasma membrane [73].

MAP17 complexes with PDZK1 and NHERF3 contributing to basal and calcium inhibition of NH₃ activity [74]. PDZK1 also regulates the solute carriers SLC15a1 (oligopeptide transporter, PEPT1) and SLC22a5 (carnitine/organic cation transporter, OCTN2) in the small intestine [75], the cystic fibrosis transmembrane conductance regulator (CFTR) and the anion exchangers of the SLC26A family, leading to its stabilization [76]. PDZK1 has also been shown to interact with AKAP10, FARP2, sodium-hydrogen antiporter 3 regulator 1, SLC22A12, SLK, SLC22A4 [77], CLCN3, cystic fibrosis transmembrane conductance regulator [78] and SLC34A3 [77]. Coupling CFTR to DRA and PAT1 results in activation of the Cl⁻/HCO₃⁻-exchange. As a consequence of transport regulation, binding of MAP17 to NHERF proteins might result in the deregulation of the intracellular and extracellular cation/anion balance, causing increased nonenzymatic oxidative stress in the cells.

On the other hand, PDZK1 interacts with CMOAT, a canalicular multispecific organic anion transporter involved in multidrug resistance. This finding was considered of particular interest because proteins containing PDZ domains are involved in the clustering and signaling pathways of membrane-associated proteins, including ion channels. Therefore, the protein cluster formed by the association of CMOAT, PDZK1, and MAP17 could play an important role in the cellular mechanisms associated with multidrug resistance, and PDZK1 may represent a new target in cancer cells resistant to chemotherapeutic agents. In line with this argument, the overexpression of PDZK1 within the 1q12-q22 amplicon is likely to be associated with the drug resistance phenotype in multiple myeloma [79].

4.2. SGLTs and Gluts transporters

The Na-dependent glucose transporter 1 (SGLT1, SCL5A1) is the main mediator of apical glucose uptake, whereas at the basolateral membrane the glucose transporter GLUT1 facilitates diffusive transport of intracellular glucose into

the bloodstream [80]. SGLT1 mRNA is present mainly in the intestine and kidney [81, 82]. Located at 22q13.1, SGLT1 is responsible for active glucose absorption, which is an energy requiring action driven by the Na⁺/K⁺ ATPase [83].

MAP17 binds and activates specific Na-dependent transport of mannose and glucose in *Xenopus* oocytes [48]. The induced transport has the functional characteristics of the Na-glucose cotransporters, the SGLTs [60]. Ectopic expression of MAP17 in human cells, tumoral or not, triggers increased absorption of glucose and mannose [47]. This transport is inhibited by phloridzin, suggesting that the increase is due to the SGLT1 transporter, corroborating the previous finding in *Xenopus* [47]. We have also found that MAP17 and SGLT1 colocalize in some tumors (Data not shown).

Previous studies demonstrated that activation of SGLT1 rescued enterocytes from apoptosis by activating PI3K [84] and that inhibition of this membrane transport with phloridzin also inhibited MAP17-dependent ROS increase and proliferation [59]. Together, these results suggest that MAP17-dependent tumorigenic properties may depend upon the activation of ROS by SGLT1 membrane transport. SGLT1, on the other hand, has been previously related to cancer [85], showing correlation with prognosis. Furthermore, silencing EGFR decreases SGLT1 significantly reducing the intracellular concentrations of glucose [86]. Despite what seems a clear functional relationship, no direct evidence for binding of MAP17 to SGLT1 in mammalian cells has been published.

In normal cells under aerobic conditions, the oxidation of glucose involves cytoplasmic glycolysis and oxidative phosphorylation by the mitochondrial electron transport chain, which produces a maximum of ATP by completely oxidizing glucose to CO₂. Under hypoxic conditions, normal cells perform anaerobic glycolysis because mitochondrial function is suppressed in the absence of O₂. Anaerobic glycolysis generates lactate from pyruvate, which is the only way to regenerate NAD⁺, the coenzyme for glyceraldehyde 3-phosphate dehydrogenase. Despite the low energy produced in anaerobic glycolysis (16 fold lower than oxidative phosphorylation), tumor cells largely rely on the conversion of glucose to lactate rather than on mitochondrial oxidation for energy production

even in the presence of high oxygen levels [87-91]. This effect is known as the Warburg effect, or aerobic glycolysis [92, 93].

Tumor cells, therefore, have mostly inhibited oxidative phosphorylation, reducing ATP production. They do not allow electrons to go through the electron transport chain all the way to oxygen, thus increasing the generation of ROS [93-95].

Thus, reduced ATP generation in mitochondria is a compromise that tumor cells make to initiate oncogenic transformation. Tumor cells must increase their access to glucose to support the high rate of glycolysis. This is achieved by an increased transport of glucose into the cells. The rate of glucose entry into tumor cells is at least 20- to 30-fold higher than in normal cells [91]. Tumor cells enhance glucose uptake via the induction of Glut1 and SGLT1, and coordinate the increased entry of glucose with increased glycolysis [91, 96, 97].

Pyruvate, but not lactate, is an effective inhibitor of histone deacetylases [98, 99]. Therefore, accumulation of pyruvate in tumor cells can potentially kill them, and aerobic glycolysis must be maintained to eliminate pyruvate and produce the lactate that will be used for essential NAD⁺ production.

On the other hand, an increased level of lactate in the cytoplasm will decrease cellular pH, potentially compromising survival. Tumor cells employ many mechanisms to prevent cellular acidification [100]. Cellular acidification results from enhanced glucose uptake and anaerobic metabolism by tumor cells. This acidic intracellular pH can be exacerbated by inefficient removal of lactic acid, CO₂ and H⁺ by deficient vasculature in the tumor [101]. Since small variations in the intracellular pH alter many biological functions such as membrane permeability, enzymatic activity, ATP maintenance and production, proliferation, migration, invasion, metastasis or drug resistance and apoptosis, the cells must regulate the intracellular pH to survive. The hypoxia inducible factor, HIF1, regulates under hypoxic conditions anaerobic glycolysis and pH homeostasis by enhancing both, the expression of glycolytic enzymes and the membrane located transporters, exchangers and ecto-enzymes [102]. The system regulating the intracellular pH in tumor cells actively export acids via the NHE1 exchanger [103-105], the monocarboxylate transporters (MCTs) [106], and, to induce cytoplasmic alkalization, transports HCO₃⁻ into the cells through Cl⁻/HCO₃⁻ exchangers and

Na⁺/HCO₃⁻ cotransports [107]. The NHE1 is also enhanced in many tumors pumping H⁺ out of the cells coupled to a transmembrane gradient. Additionally, H⁺/lactate-coupled transporters, carbonic anhydrases, could facilitate H⁺ efflux, preventing acidification of the cytoplasm [108].

Most of these membrane transporters could be regulated by MAP17 levels and localization through NHERF proteins binding (Figure 3B). Therefore, the MAP17/NHERF complexes could be an intracellular pH/acidosis detoxifying system parallel to carbonic anhydrases. It is possible that MAP17/NHERFs-dependent system be also induced under hypoxic conditions maintained through further malignization. However, the fact that levels of MAP17 rise with the stage of the tumor suggests that may be more related to continuous selection of cells with higher pH detox capabilities. Further experimental validation is necessary to clarify these hypotheses.

By activating MAP17, cells will insure increased membrane localization of internal pH detoxifying transporters. Several oncogenes could activate MAP17 expression [56] promoting MAP17 increase along with increased metabolism. Unspecific membrane transporter inhibitors, such as furosemide, produce similar effects on cellular proliferation than phloridzin, an SGLT inhibitor, suggesting the functional relationship between SGLT and the pH regulation [59]. Thus, enhanced membrane transport to prevent acidification may be regulated by MAP17 through NHERF binding allowing SGLT1 increase and anerobic glycolysis activation in tumor cells.

However, it is also possible that secondary effects of an increase in glucose and mannose, such as glycosylation and mannosylation of proteins might alter properties of the cells, including oxidative stress or tumorigenic properties, thus contributing to the MAP17-induced tumoral phenotype.

5. MAP17 and cancer therapy

Treatment of melanoma cells with inhibitors of Na⁺-coupled cotransporters leads to an inhibition of a ROS increase and a decrease in the malignant cell behavior in MAP17-expressing clones [59]. These changes could result in a new therapeutic approach by nonspecifically blocking uphill transport in tumor cells.

However, the rapid adaptation of the cells to these pharmacologic interventions and possible toxic effects need to be better studied.

A low level of ROS is indispensable in several physiological processes of the cell, including proliferation and apoptosis, or cell death [109]. A mild increase in ROS has been shown to activate signaling cascades which can seriously influence the regulation of cell growth and tumorigenic processes [4, 10, 11, 16, 31, 42, 43, 57, 110]. However, a further increase in ROS levels raises oxidative stress and creates a potentially toxic environment for the cell. In normal physiological conditions, a balance between ROS generation and oxidative defenses exists in the cell. In these defenses, endogenous antioxidant enzymes play a significant role. Superoxide dismutase (SOD) and catalase (CAT) that act on O_2^- and H_2O_2 , respectively, glutathione peroxidases (GPXs) that use glutathione as a cosubstrate, peroxiredoxins and thioredoxins, are in a delicate balance with oxidative inputs [1, 110]. Although many cells can tolerate limited doses of ROS, when the balance tips further in favor of ROS, programmed cell death becomes a certainty [111]. Cellular detoxification enzymes cannot neutralize excessive ROS that alter the chemical environment in the cells, especially within the mitochondria, and launch the cell death program.

Therefore, we can hypothesize that tumors expressing high levels of MAP17 and producing ROS can benefit from therapies that increase oxidative stress, such as cisplatin and radiotherapy, doxorubicin or camptothecin, which have redox-mediated activity [112] on tumor cells without affecting healthy tissues [113]. Tumors with high MAP17 can also benefit from combined therapies using cytotoxic agents and increased oxidative stress. The first attempt to employ pro-oxidant agents in vivo was reported by Nathan and Cohn in 1981. Using glucose oxidase as an H_2O_2 precursor, they obtained a significant decrease of tumor growth [114, 115].

Because cells can develop adaptive responses to ROS, primarily based on the increase of detoxifying enzymes [8], we can hypothesize that the inhibition of classical oxidative stress detoxifying enzymes can also increase the efficacy of certain antitumor therapies that increase ROS, at least in tumors that overexpress MAP17. However, the delicate balance between oxidative stress, cancer and cell

death make it necessary to devise new experimental tests and develop a more complete understanding of these processes.

6. CONCLUSIONS

In summary, the observation that MAP17 is overexpressed in human carcinomas indicates that MAP17 can be a good marker for tumorigenesis and for malignant progression. The results indicate that this protein is likely to play an important role in carcinogenesis and likely in the response to certain therapies related to oxidative stress. On the other hand, the overexpression of PDZK1 within the 1q12-q22 amplicon is likely to be associated with the drug resistance phenotype in multiple myeloma [79]. All of these data suggest that the uphill transport and SGLT1-dependent glucose uptake might be important players in the correct balance of ROS in cells, indirectly regulating the ability to select more aggressive tumor cell populations. Therefore, a deeper understanding of this system might be of interest to the cancer field.

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Figure legend

Figure 1: Representative pictures of human tumors overexpressing MAP17. A) Colorectal carcinoma. B) Undifferentiated cervical carcinoma. C) Mammary carcinoma. D) Lung adenocarcinoma. Pictures show tumor cells, characterized by nuclear displasia, heavily stained for MAP17 expression. Non tumoral cells forming the stroma, show no staining.

Figure 2: Overexpression of MAP17 in human tumors. Graphs show overexpression of MAP17 mRNA in tumors compared with non-tumoral tissue in a large-scale microarray data set from different studies (www.oncomine.com). A) From [116]. B) From [117]. C) From [118]. D) From [119]. E) From [120]. F) From [120].

Figure 3: A) Scheme of the possible functional role of MAP17 as an anchor for PDZK1-membrane transporter complexes. B) Scheme of hypothetic functional relationship between SGLT1 and MAP17 through NHERF. SGLT1 increase in tumor cells to respond to high glucose demand, while MAP17 increase to regulate the subsequent intracellular acidification through NHERF binding and localizing pH detoxifying enzymes in the membrane.

Abbreviations

PDZ, Post-synaptic density 95, Disc large, Zonula occludens 1

PDZK1, PDZ domain-containing 1

CLAMP, c-terminal linking and modulating protein;

CAP70, CFTR-associated protein, 70-KD

MAP17, Membrane associated protein 17 KD

NHERF, Na⁺/H⁺ exchanger regulatory factor

IKEPP, Intestinal and kidney enriched PDZ protein

SGLT, Na⁺/glucose cotransporter