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REVIEW ARTICLE

Evaluation of potential interactions between the metastasis-associated protein S100A4 and the tumor suppressor protein p53

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Abstract Metastasis is a complex cascade of events involving a finely tuned interplay between malignant cells and multiple host factors. The transition from benign tumor growth to malignancy is manifested by the ability of tumor cells to traverse tissue barriers and invade surrounding tissues. Among a multitude of factors playing a role, the small calcium-binding protein S100A4 has been found to add to the invasive and metastatic capacity of cancer cells. However, the exact molecular function or mechanism by which S100A4 exerts its putative metastasis-promoting effects has not been fully elucidated, and the protein is most likely involved in several aspects of tumor progression. Several studies have recently described a direct interaction and/or reciprocal influence between S100A4 and the tumor suppressor protein p53. This corresponds to reports linking p53 to other S100-family members, especially S100B. The consequences are intriguing, connecting the metastasis-promoting protein S100A4 to the large set of important p53-mediated functions, with broad potential importance in cancer development and metastasis. In this review we emphasize the studies involving p53 and S100A4, elucidating and comparing reported results and conclusions.

The S100-protein family

In humans more than 20 proteins of the S100 family are reported. The first family members were discovered in brain tissue by Moore and given the name S100 because they were soluble in 100% saturated ammonium sulfate (Moore 1965). The S100 proteins share a high degree of sequence homology and most have a similar 3D structure, with the majority of family members existing as anti-parallel packed homodimers (while cases of heterodimers or oligomers are known). Each subunit contains two distinct EF-hand helix–loop–helix calcium-binding domains, one common to all EF-hand proteins on the C-terminal part, and one specific to this protein family located at the N terminus (Zimmer et al. 2003). Upon calcium binding, S100A4 and most other dimeric S100 proteins, undergo a relatively large conformational change which opens up a hydrophobic binding pocket allowing interaction with target molecules and thereby modulation of their functions (Pathuri et al. 2008; Tarabykina et al. 2007). The S100-proteins have been characterized as multifunctional signaling proteins having a broad range of intracellular and extracellular roles. The biological diversity is determined both by their cell- and tissue-specific expression and by their different biochemical properties (lipid- and metal-binding, molecular conformation, oligomerization status, heterodimerization with other family members, posttranslational modifications etc.) (Donato 2003; Santamaria-Kisiel et al. 2006; Marenholz et al. 2004; Haugen et al. 2008).

Intracellular functions of S100 proteins include, amongst other, regulation of protein phosphorylation, enzyme activity, and transcription factors, modulation of cytoskeleton constituents, cell growth and differentiation, and calcium homeostasis. The existence of multiple S100 protein targets may explain the involvement of the S100 proteins in a large variety of cellular activities such as neurite outgrowth, cell–cell communication, cell growth- and division, cell structure, energy metabolism, contraction, motility,

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intracellular signaling, and angiogenesis (Donato 2003; Santamaria-Kisiel et al. 2006). Several of the S100 proteins have been implicated in the progress of various diseases including cardiomyopathy, neurodegenerative disorders, inflammatory responses and association with different stages and types of cancer; S100A2, S100A4, S100A6, S100A7, S100A11, S100P and S100B are postulated to play a role in the progression of human cancer (Emberley et al. 2004; Marenholz et al. 2004; Garrett et al. 2006; Heizmann et al. 2002; Salama et al. 2008). While not common in clinical practice, S100B has been evaluated as a diagnostic marker and for therapeutic monitoring in malignant melanoma (Hauschild et al. 1999; Schultz et al. 1998; Hamberg et al. 2003). Even though the precise role of S100 proteins in the development and promotion of cancer remains unclear, it is conceivable that the different S100 proteins have several functions and interact with multiple proteins during tumorigenesis and metastasis (Salama et al. 2008). To date more than 90 potential protein targets have been identified for the S100 proteins (Santamaria-Kisiel et al. 2006).

The metastasis-associated protein S100A4

Numerous reports have convincingly linked the expression of S100A4 to the invasive and metastatic capacity of cancer cells (for review see: Garrett et al. 2006; Helfman et al. 2005; Sherbet and Lakshmi 1998). S100A4 is a small (101aa) acidic 11.5-kDa protein, originally cloned from various cell systems and given different names (mts1, p9Ka, FSP1, CAPL, calvasculin, pEL98, metastasin, 18A2 and 42A) (Garrett et al. 2006). While the S100A4 protein is not tumorigenic per se, elevated expression was found in a multitude of different cancers and it soon became apparent that the presence of S100A4 was coupled with increased metastatic capacity. In brief, expression of S100A4 in non-metastatic cell lines resulted in a more metastatic phenotype (Davies et al. 1993; Grigorian et al. 1996), while decreased expression of the protein led to cells with less metastatic capacity (Maelandsmo et al. 1996; Takenaga et al. 1997). In studies of transgenic animals, expression of S100A4 was associated with more aggressive and invasive tumors and increased metastatic potential (Ambartsumian et al. 1996; Davies et al. 1996). Furthermore, enhanced expression has been associated with poor prognosis in a number of human cancers, and S100A4 has been proposed as a potential prognostic marker predicting metastasis and survival in the examined cohorts (Andersen et al. 2004; Rudland et al. 2000; Helfman et al. 2005). In spite of all data, the biological functions and molecular mechanisms by which S100A4 exerts its putative metastasis-promoting effects are largely unknown, and the protein is most likely involved in several aspects of tumor progression. Possible explanations for the multifunctionality includes that the protein is localized in different subcellular compartments and probably has different binding partners in the cytoplasm, the nucleus, and the extracellular space.

S100A4 interacting proteins and possible biological functions

In the cytoplasm S100A4 has been documented to co-localize or co-sediment with different cytoskeletal proteins [F-actin (Mandinova et al. 1998), non-muscle myosin/myosin-IIA (Li and Bresnick 2006; Dulyaninova et al. 2005; Li et al. 2003; Kriajevska et al. 1994, 1998), non-muscle tropomyosin (Takenaga et al. 1994; Takenaga and Masuda 1994) and liprin beta1 (Kriajevska et al. 2002)], implying a possible mechanistic role in cell shape, motility and thus invasion. Interactions with non-structural proteins like p37 (Dukhanina et al. 1997), S100A1 (Wang et al. 2000; Tarabykina et al. 2000), methionine aminopeptidase-2 (Endo et al. 2002) and CCN3 (Li et al. 2002) have also been suggested to play a role for the metastasis promoting effects of S100A4.

Similar to some other S100 proteins, S100A4 is also localized extracellularly (Pedersen et al. 2004; Ambartsumian et al. 2001), possessing a wide variety of extracellular effects, including sensitization of cancer cells to IFN-γ mediated apoptosis (Pedersen et al. 2004), stimulation of angiogenesis (Ambartsumian et al. 2001; Semov et al. 2005) and neurite outgrowth (Novitskaya et al. 2000). Previous research by us and others has demonstrated an association between the expression of S100A4 and the activity of several matrix metalloproteinases (MMPs). MMPs and their endogenous inhibitors (tissue inhibitors of MMPs; TIMPs) have been shown to correlate with in vitro invasiveness and clinical outcome in several malignancies, thus possibly explaining some of the S100A4 induced effects on invasive and metastatic capability (Schmidt-Hansen et al. 2004a, b; Bjornland et al. 1999; Saleem et al. 2006; Mathisen et al. 2003; Andersen et al. 1998). Expression of MMPs has also been linked back to increased levels of S100A4 in non-malignant conditions like rheumatoid arthritis and osteoarthritis (Klingelhofer et al. 2007; Oslejskova et al. 2008; Senolt et al. 2006).

While strong data indicate that release of S100A4 into the tumor microenvironment is a crucial factor in the metastatic process, the signal transduction pathways responsible for S100A4-induced effects are only starting to become unraveled. Extracellular S100A4 has been shown to activate NF-κB (Pedersen et al. 2004; Schmidt-Hansen et al. 2004b), and in recent work, we have demonstrated this S100A4-induced effect to go through the classical

NF-κB activation pathway (Boye et al. 2008; Grotterød et al. manuscript in preparation).

In summary, both intracellularly and extracellularly localized S100A4 seem to interact with proteins involved in tumor progression and metastasis formation.

p53: "the guardian of the genome"

The tumor suppressor protein p53 plays a pivotal role in the maintenance and regulation of normal cellular functions through the induction of cell cycle arrest, DNA repair or apoptosis in response to a variety of cellular stress signals and DNA damage (Levine 1997; Aylon and Oren 2007; Joerger and Fersht 2008). Mutations in the *TP53* gene are found in almost all types of human cancer with a frequency ranging from 20 to 60% (Hollstein et al. 1994).

Owing to its essential role in cell cycle control, p53 is subjected to a complex regulation, and the biochemical activity of p53 as a transcription factor is adjusted by phosphorylations and acetylations, as well as modulation of protein stability, degree of oligomerization, nuclear translocation, and interactions with other components of the transcriptional machinery (Hupp 1999; Chene 2001). The resulting active transcription factor has the capacity to regulate the expression of numerous downstream genes, although some apoptotic activities seem to be transcriptionally independent. A large set of proteins that directly binds to, and acts on p53 have been identified (reviewed in Levine 1997; Joerger and Fersht 2008).

p53 can be divided into different functional domains (Fig. 1). A transactivation and a proline-rich domain (∼residues 1–50 and 61–97, respectively) are present at the N-terminus. The DNA-binding domain (DBD) is located in the middle of the protein (102–292). The C-terminal region (CT) contains several functional domains; three nuclear localization signals (NLS I, the most conserved at 313–322) (Shaulsky et al. 1990; Liang and Clarke 1999), the tetramerization domain (TET, ∼320–356) and the negative regulatory domain (NRD, ∼367–393) (Chene 2001; Hupp 1999). In normal unstressed cells, wild type p53 levels are low with the protein predominantly present in a monomeric state (Poon et al. 2007). However, in its active DNA-binding conformation p53 is present as a tetramer (Weinberg et al. 2004; Rajagopalan et al. 2008; Halazonetis and Kandil 1993; Chene 2001). The TET domain has also been shown to be essential in protein–protein interactions, post-translational modifications and p53 degradation (Itahana et al. 2008; Chene 2001). In addition, the tetramerization domain harbors a nuclear export signal (NES, 340–351) (Stommel et al. 1999) that can be masked by formation of tetramers, indicating that p53 cellular localization could be regulated by changes in its quaternary structure (Liang and Clarke 2001).

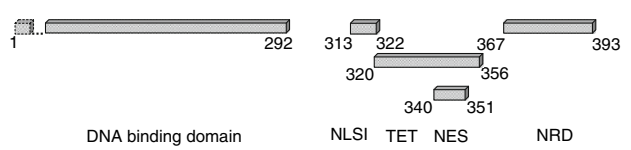

Fig. 1 A schematic overview of major domains of the p53 protein. NLS, nuclear localization signal; TET, tetramerization domain; NES, nuclear export signal; NRD, negative regulatory domain

Interactions between S100 proteins and p53

Previously, reciprocal interactions between p53 and various S100 family members have been demonstrated, the most extensively studied being S100B (Baudier et al. 1992; Rustandi et al. 1998, 2000; Delphin et al. 1999; Fernandez-Fernandez et al. 2005; Wilder et al. 2006; Lin et al. 2001, 2004; van Dieck et al. 2009). The interaction with S100 proteins has been reported to affect several aspects of p53-structure and -function, such as phosphorylation and acetylation, and subsequent modulation of its subcellular localization and transcriptional activity. However, the downstream effects vary, and are in addition disputed (Ikura and Yap 2000; Fernandez-Fernandez et al. 2008; Donato 2003).

Physical interactions

In a paper from 2001, Grigorian et al. (2001) for the first time claimed a physical and functional interaction between S100A4 and p53. They demonstrated by several in vitro approaches that S100A4 binds to the extreme end of the p53 C-terminal regulatory domain, mapping the area to within aa 360–393. This binding led to inhibited in vitro phosphorylation of both the C-terminal peptide and the full length p53 by protein kinase C (PKC). The interaction was further found to affect the DNA binding capacity of p53 in an experimental setting. Few studies have followed up this interesting work. However, by using an optical biosensor, an interaction between immobilized S100A4 and p53 was reported (Chen et al. 2001). The binding constant, Kd, was found to be in the same range as previously reported for the interaction between p53 and S100B (Delphin et al. 1999). Due to the methods used it is difficult to rule out whether S100A4 existed in a monomeric or dimeric form on the biosensor matrix, and it is furthermore surprising that the interaction between p53 and S100A4 was independent of calcium (Chen et al. 2001). The presence of calcium has been considered as a prerequisite for S100A4 interactions (Dutta et al. 2002; Tarabykina et al. 2007), and has been demonstrated to be essential for the interaction between p53 and S100B/S100A2/S100A6 (Fernandez-Fernandez

et al. 2005; Baudier et al. 1992; Delphin et al. 1999; Rustandi et al. 2000; Scotto et al. 1998; Mueller et al. 2005; Slomnicki et al. 2009).

In a later study, Fernandez-Fernandez et al. (2005) found by fluorescence anisotropy and equilibrium sedimentation experiments (AUC) that S100A4 binds to p53CT (residues 293–393), however, with a high Kd required. The authors proposed the strong binding of S100A4 to p53 found in (Chen et al. 2001) to be caused by incorrect folding of S100A4 on the biosensor matrix, somehow exposing novel binding sites for p53 that are not accessible in solution (Fernandez-Fernandez et al. 2005). In addition, only a low binding of S100A4 to NRD (eight times more weakly than S100B), the region claimed to be the binding site by Grigorian et al. (2001) was reported. In contrast, S100A4 was measured to bind tighter than S100B to NES- (340–351) and TET- (325–355) peptides, while not at all to NLS (peptide 305–322).

Importantly, in this study the p53 TET-peptide bound by S100A4 and S100B was in the lower oligomerization state (monomer/dimer), and tetramerization of the peptide prevented the interaction (Fernandez-Fernandez et al. 2005). Partly in discrepancy with these data, the same group reported in a very recent publication that the interaction between S100A4 and p53CT monomer is very weak not forming a stable complex, when investigated by analytical size-exclusion chromatography and multi-angle light scattering. Furthermore, dimeric and tetrameric forms of p53CT did not form complexes with S100A4, and only a weak interaction was obtained using full-length tetrameric p53. S100B was found to interact with both p53CT monomers, dimers and tetramers, as well as with full length p53, though weakly with the latter (van Dieck et al. 2009). The recent results are unfortunately not discussed more thoroughly with respect to the data in (Fernandez-Fernandez et al. 2005).

When comparing the interactions between p53 and the two S100 proteins marked variations have thus been reported. First, opposed to S100A4, S100B binds different peptides from within the whole p53 C-terminal region (residues 293–393) shown by fluorescence anisotropy and AUC (Fernandez-Fernandez et al. 2005) and more specifically it binds strongly to the negative regulatory domain (NRD, 367–393) shown by NMR and plasmon resonance (Delphin et al. 1999; Rustandi et al. 1998, 2000). Second, when using recombinant p53CT the binding of S100B was 40 times tighter than for S100A4, the difference in binding strength reflecting synergistic effect of the larger number of binding sites for S100B. The reported data for both S100 proteins fit a model where two dimers bind sequentially to one p53CT molecule (Fernandez-Fernandez et al. 2005). Opposed to this, a stoichiometry of one dimer of S100A4 or S100B bound to a monomer of p53, two dimers of S100B in a complex with a tetramer of p53CT and four dimers of S100B binding to a tetramer of full length p53 were recently reported (van Dieck et al. 2009). The same study also suggested a new potential binding site for S100 proteins, localized in the transactivation domain (peptide 1–57) using fluorescence anisotropy (van Dieck et al. 2009).

For two other S100 proteins, S100A1 and S100A2, binding to the negative regulatory domain (NRD) has been reported (Garbuglia et al. 1999; Mueller et al. 2005; Fernandez-Fernandez et al. 2008). Recently, binding to the p53 tetramerization domain (TET) was claimed to be a general feature of the S100 family members, while a subset of the proteins in addition bind NRD, the two groups exemplified by S100A4 and S100B, respectively. In this study, S100A1, S100A2, S100A6 and S100A11 were overexpressed and found in vitro to bind the TET domain, affecting p53 oligomerization (Fernandez-Fernandez et al. 2008). Bearing this in mind, a balance of actions of different S100 proteins within a cell has been suggested to determine function of the respective S100 family member (Salama et al. 2008). Moreover, variation in the length and composition of the interaction interface and divalent cation-binding capacity may significantly alter binding of different S100 proteins and certainly contribute to target specificity and therefore distinct functions (Bhattacharya et al. 2004; Donato 1999; Wilder et al. 2006; Zimmer et al. 2003; Pathuri et al. 2008; Malik et al. 2008; Malashkevich et al. 2008; Dutta et al. 2008).

In conclusion, an interaction between S100A4 and p53 has been shown in different in vitro studies, but importantly, and little discussed, is the reported discrepancy in binding specificities and molecular conditions.

Functional interactions

Despite a number of in vitro and in vivo studies that have addressed the question, the net effect of how S100B binding alter p53 function is under debate (Ikura and Yap 2000). Even more vaguely described is a possible functional effect of S100A4/p53 interaction. Based on the finding that both S100B and S100A4 bind to the TET domain of p53, inhibiting its oligomerization, and for S100B promoting oligomer disassembly, suggestions have been made that these S100-proteins could regulate localization and activity of p53 (Fernandez-Fernandez et al. 2005; van Dieck et al. 2009; Baudier et al. 1992; Lin et al. 2001). The intracellular transport of p53 depends on its oligomerization, and there are indications that p53 enter the nucleus more efficiently in monomeric than in tetrameric form (Liang and Clarke 2001). Based on data from in vitro studies, Scotto et al. (1998, 1999) hypothesized that in glial cells S100B could synergize with p53-dependent growth

arrest and apoptosis by favoring p53 nuclear translocation and activation of G1 checkpoint control. Theorized by Fernandez-Fernandez, S100B could once inside the nucleus bind to the NRD potentially protecting p53 from modifications needed for degradation. While in contrast, increased nuclear transport by S100A4, which not binds NRD, would lead to increased targeting of p53 for degradation (Fernandez-Fernandez et al. 2005). Chen et al. (2001) on the other hand, argues for a cytoplasmic sequestration of p53, based on the general view that S100A4 is mostly reported outside the nucleus. The latter finding is however, challenged in recent studies (Flatmark et al. 2003; Kikuchi et al. 2006; Berge et al. 2010) where nuclear expression of S100A4 has been reported.

Biological consequences

The regulation of wt p53 nuclear translocation and accumulation is complex and amongst other dependent on PKC phosphorylation of the C-terminal end of the protein (Scotto et al. 1999; Hupp 1999; Giaccia and Kastan 1998). As noted above, two studies found S100B to favor nuclear accumulation and activity of p53 (Scotto et al. 1998, 1999). Without conclusion regarding downstream effects, S100B was shown to bind p53 and inhibit in vitro phosphorylation, hampering oligomerization and increasing disassembly of p53 tetramers (Baudier et al. 1992; Delphin et al. 1999). In other studies S100B-mediated inhibition of PKC phosphorylation and tetramer formation led to decreased p53 DNA binding and transcriptional activity (Lin et al. 2001, 2004; Rustandi et al. 2000; Wilder et al. 1998, 2006). S100B was therefore suggested to inhibit p53 function and thus contribute to cancer progression (Lin et al. 2001). Trying to explain such conflicting data, a theoretical model was recently put forward where, depending on the concentration of p53 and the given member of the S100 family, interaction between the proteins would alter the balance between monomer and tetramer form of p53 (van Dieck et al. 2009). Of notice in this respect, the presence of S100A6 was in vitro shown to coincide with more efficient nuclear accumulation of p53 under stress conditions (Slomnicki et al. 2009). In addition, both S100A2 and S100A6 are shown to modulate p53 transcriptional activity (Mueller et al. 2005; Slomnicki et al. 2009).

Interestingly, Grigorian et al. (2001) found S100A4 to inhibit PKC-mediated phosphorylation of the C-terminal part of p53. Therefore, it was speculated whether S100A4, in a similar manner as S100B could be an inhibitor of p53 function. However, by the use of two different methods the authors found a complex S100A4-mediated regulation of p53 transactivation. The co-transfection of S100A4 and a luciferase reporter plasmid under the control of synthetic p53-responsive elements from promoter regions of given genes led to inhibition of p21- and a slight increase in Bax transcription (1.5- to 3-fold). Similarly, in a system where S100A4 expression was induced by addition of doxycycline, the expression of several genes were up-regulated (e.g. Bax), MDM2 was down-regulated initially and then later up-regulated, and p21 was inhibited. Although the observed S100A4-mediated alterations are small, the authors draw the general conclusion that S100A4 is able to modulate p53 transactivation. The functional consequence of this transcriptional regulation is complicated by the fact that several of the examined p53-responsive genes are pro-survival, whereas others are pro-apoptotic, and the S100A4-mediated responses on these two classes of effect molecules do not necessary correlate. The reason for these seemingly opposite effects of S100A4 expression on the p53-regulated genes is not elaborated. In addition, the modulating effect of S100A4 on p53 transcriptional activity seems in some cases to depend on the proliferation rate or the density of the cell cultures. Such parameters may be difficult to standardize and it may therefore be challenging to repeat and confirm the observed S100A4 mediated effects in other cell systems.

In the same study, the authors also reported apoptotic cell death when S100A4 was transfected into *TP53* wt cell lines, while co-transfection of the anti-apoptotic gene *bcl-2* rescued the clones. In *TP53* mutated cell lines however, *S100A4* transfection was successful (Grigorian et al. 2001).

Based on these findings, Grigorian et al. (2001) postulated that S100A4, through interaction with p53, may induce apoptotic cell death in tumor cells harboring wt p53, contributing to selection of cells with mutated *TP53* and increased metastatic potential. In a recent work, we argue against this hypothesis as simultaneous expression of S100A4 and wild type p53 in a number of cell lines and colon cancer tissue samples was observed (Berge et al. 2010). The best example being the human colon cancer cell line HCT116 which is verified wt *TP53*, and still expresses high levels S100A4, while the isogenic *TP53* knock-out variant (*TP53*−/−) (Bunz et al. 1999) exhibit very low levels (Daoud et al. 2003; Mann and Hainaut 2005; Berge et al. 2010). In agreement with our findings, co-expression of wt p53 and S100A4 was also reported in the lung cancer cell line A549 (Orre et al. 2007). In principle, these observations alone contradict the above described theory of a mutually exclusive relationship between *TP53* mutational status and S100A4 expression.

Reciprocal influence between S100-proteins and p53

Utilizing this HCT116 cell system, we investigated reciprocal effects of S100A4 and p53 regulation, though without testing direct interactions between the two proteins. Experimental up or down regulation of S100A4 exerted no

effects on p53 protein expression, and did not influence the stabilization of p53 caused by UV-induced DNA damage. Similarly, alteration in p53 level did not support mutual regulatory effects between the two proteins (Berge et al. 2010). Our findings are in agreement with a recent report showing no alteration in S100A4 expression after oxidative stress induced up-regulation of p53 in the HCT116 wt *TP53* cell line (Orre et al. 2007). Similarly, after treatment of both the two isogenic HCT116 cell lines with Topotecan and Aminothiol, which leads to DNA damage and p53 accumulation, gene expression of S100A4 was found to be independent of drug treatment (Daoud et al. 2003; Mann and Hainaut 2005). These results are in contrast to suggestions made in both articles of a close association between S100A4 expression and p53 function, based on the variation in basal S100A4 expression observed in the wt *TP53* and *TP53*−/− lines (Mann and Hainaut 2005; Daoud et al. 2003). Conversely, a lower level of S100A4 was observed after ionizing radiation of the lung carcinoma cell line A549, treated with si-RNA constructs against p53 (Orre et al. 2007).

In contrast to data for S100A4, S100B was in malignant melanoma cell lines shown to down regulate p53 and inhibit p53 mediated cellular effects, whereas restored levels and function were attained in S100B knock down cells (Lin et al. 2004). Furthermore, overexpression of S100B effectively prevented induction of p53, p21 and MDM2 in co-transfection assays and in response to bleomycin induced DNA damage (Lin et al. 2001). Together, these findings imply that elevated levels of S100B could inhibit p53 functions and contribute to cancer progression (Lin et al. 2001). Hence, although very similar experimental conditions were applied in the referred studies on S100B and in our in vitro models investigating S100A4, none of the profound effects were observed that would be expected if S100A4 interacted with, or regulated p53 in the same manner as S100B.

Without being investigated further, regions with 85% homology to a p53-binding element has been reported in the *S100A4* promoter and transcriptional regulation by p53 thus postulated (Parker et al. 1994). This is similar to a reported p53 regulatory effect of S100A2 expression by means of a p53 binding sites in the *S100A2* promoter (Tan et al. 1999). Likewise, in the *S100B* promoter regions exactly matching the consensus p53-binding motif are found, and p53 is suggested to upregulate S100B as part of a negative feedback loop, analogous to hdm2 (Lin et al. 2004; Slomnicki et al. 2009).

Potential in vivo effects of S100A4 and p53 interactions

Although a direct interaction between p53 and S100A4 has been demonstrated in vitro, convincing data showing that this interaction also exists in vivo has to our knowledge so far not been presented. Furthermore, no direct evidence has been published showing that the various S100A4 binding partners suggested from in vitro experiments are of importance for the biological effects induced by S100A4. In fact, using a yeast two-hybrid system has not identified an1 interaction between S100A4 and reported in vitro targets, except S100A1 (Wang et al. 2000; Tarabykina et al. 2000).

The only published result suggesting a direct contact between S100A4 and p53 in vivo involve co-immunoprecipitation from cells harboring mutant p53 (Grigorian et al. 2001). Interestingly, such co-immunoprecipitation has so far not been successful in cells with wt p53. Furthermore, the binding of S100A4 to the C-terminus of p53, with potential implications for DNA binding, p53 phosphorylation by PKC or downstream target activation, has been demonstrated in vitro, but the in vivo relevance is still to be investigated. The idea that S100A4 and wt p53 is mutually exclusive is appealing, but the hypothesis has until recently not been investigated in a clinical material. For such a mechanism to be considered relevant, one would expect the favored combinations in tumor samples to be either wt *TP53*/S100A4 negative or mutated *TP53*/S100A4 positive. We have recently found this not to be the case for a number of primary colorectal carcinoma biopsies and CRC cell lines (Berge et al. 2010). In the same cohort of CRC patients, nuclear expression of S100A4 was an independent predictor of patient demise (Boye et al. manuscript in preparation). The nuclear function of S100A4 is completely unknown, but based on the observation that p53 and S100A4 co-localize in the same nucleus it might be speculated whether the two proteins interact and that the formed complex further support tumor invasion and metastasis. This hypothesis remains however to be investigated.

Following the in vitro data coupling S100A4 to p53-dependent apoptosis (Grigorian et al. 2001), the formation of spontaneous tumors in S100A4−/− mice was suggested to be caused by impaired interaction between the proteins. In the S100A4−/− mice 10% of the 10 to 14-month old animals developed spontaneous tumors, a characteristic feature for strains with inactivated tumor suppressor genes. By immunohistochemistry (IHC) the tumors were found to be p53 positive, and thus claimed to express elevated levels of mutated or functionally altered p53 protein (EL Naaman et al. 2004). Numerous reports have, however, demonstrated detection of wt p53 by IHC, and it has been suggested that the protein may be abnormally sequestered in the cytoplasm in a subset of human tumors (Liang and Clarke 2001; Florenes et al. 1994; Rassidakis et al. 2005; Matsushima et al. 1994). For drawing unambiguous conclusions on *TP53* mutational status it is therefore necessary to verify IHC data by molecular analysis (Soussi 2007).

In the same study, reduced frequency of apoptosis in the spleen of the S100A4−/− animals compared to the

wild-type animals after whole-body gamma-irradiation was described. Also diminished transcriptional activation of the p53 target genes p21 and bax (as measured by IHC), was reported while no information of the status of other genes in the p53 regulatory system are available (EL Naaman et al. 2004). Based on these observations the authors claim that the spontaneous tumors in the S100A4−/− mice could be a result of functional destabilization of the *TP53* tumor suppressor gene caused by disruption of the S100A4-p53 interaction in the *S100A4* null mice (EL Naaman et al. 2004). This is an interesting hypothesis, but it is also worth noticing that p53 is only one element in a network of pathways that link genotoxic stress and growth control. Thus loss or gain of function in other genes can be equivalent to p53 inactivation (Soussi and Beroud 2001), and such possibilities seem not to be fully evaluated in the referred study.

A opposite conclusion of interaction between the proteins was put forward based on an immunohistochemical analysis of 94 primary lung adenocarcinomas, where a strong inverse relationship was found between S100A4 and nuclear p53 expression (presumed a mutated form) (Matsubara et al. 2005). In contrast, no correlation was observed between S100A4 and p53 expression in cohorts of 103 stage I non small cell lung cancer (NSCLC) patients (De Petris et al. 2008) or 349 stage I and II breast cancer patients (Rudland et al. 2000).

In conclusion, and with the limitations and inconsistencies presented, it is our opinion that a direct interaction in vivo between the proteins or a contact in the form of a "functional destabilization" is neither verified nor denied.

Conclusion

S100A4 has been found to physically interact with p53 in vitro, however, the downstream effects are not satisfactorily elaborated for further conclusions to be drawn regarding biological significance of the interaction. Moreover, a possible reciprocal regulation between the two proteins is not confirmed. Even without conclusive evidence of an in vivo interaction between the proteins, it has become consensus in the literature that p53 is an important intracellular target for S100A4. Although an interaction between the proteins may exist, we can, based on our investigations in colorectal cancer, argue against the hypothesis of an apoptosis promoting effect of wt p53/S100A4 expression (Berge et al. 2010).

Regardless of any interaction between S100A4 and p53, S100A4 has convincingly been demonstrated to promote development of a metastatic phenotype. As reported in numerous studies, the metastasis promoting properties of S100A4 can be caused by direct interactions with other targets such as cytoskeletal proteins, or induction of other metastasis promoting factors for example MMPs. In conclusion, the existence of a biological interaction between S100A4 and p53, or that such an interaction is of importance in human cancer development or S100A4 enhanced metastasis formation, has yet to be established.

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