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Involvement of stromal p53 in tumor-stroma interactions

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Abstract

p53 is a major tumor-suppressor gene, inactivated by mutations in about half of all human cancer cases, and probably incapacitated by other means in most other cases. Most research regarding the role of p53 in cancer has focused on its ability to elicit apoptosis or growth arrest of cells that are prone to become malignant owing to DNA damage or oncogene activation, i.e. cell-autonomous activities of p53. However, p53 activation within a cell can also exert a variety of effects upon neighboring cells, through secreted factors and paracrine and endocrine mechanisms. Of note, p53 within cancer stromal cells can inhibit tumor growth and malignant progression. Cancer cells that evolve under this inhibitory influence acquire mechanisms to silence stromal p53, either by direct inhibition of p53 within stromal cells, or through pressure for selection of stromal cells with compromised p53 function. Hence, activation of stromal p53 by chemotherapy or radiotherapy might be part of the mechanisms by which these treatments cause cancer regression. However, in certain circumstances, activation of stromal p53 by cytotoxic anti-cancer agents might actually promote treatment resistance, probably through stromal p53-mediated growth arrest of the cancer cells or through protection of the tumor vasculature. Better understanding of the underlying molecular mechanisms is thus required. Hopefully, this will allow their manipulation towards better inhibition of cancer initiation, progression and metastasis.

Keywords

Stroma; cancer; p53; cancer-associated-fibroblasts; SDF-1

1 Introduction

p53 is a major tumor-suppressor gene, mutationally inactivated in about half of all human cancer cases [1]. In most of the other half of cases, which progress with a wild type (wt)^{*} p53 gene, p53 function is probably incapacitated by disruption of the p53 activation pathway or of p53 downstream effectors. In normal, unstressed cells, p53 is largely kept in check through interactions with its major regulator, Mdm2, leading to the rapid proteasomal degradation of p53 [2]. Genotoxic insults, activated oncogenes and a variety of additional stress conditions upregulate p53, usually through disruption of the p53-Mdm2 interaction. Following its activation, the p53 protein undergoes post-translational modifications, causing its stabilization and enhanced nuclear accumulation. In the nucleus, functioning as a transcription factor, p53

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^{*}Abbreviations: CAF: cancer-associated fibroblast, ECM: extracellular matrix, IL: interleukin, LOH: loss of heterozygosity, MMP: matrix metalloproteinase, SDF-1: stromal derived factor 1, Tsp-1: thrombospondin-1, wt: wild type.

orchestrates the concerted response of hundreds of genes [3]. The endpoint of this response is either resolution of the stress that has induced p53 activation (e.g. by repairing the damaged DNA) or induction of apoptosis or cellular senescence of the cell in which p53 has been activated, thus preventing the proliferation of cells that might spawn cancer. Induction of differentiation and accelerated DNA repair are additional p53 activities that conceivably contribute to inhibition of malignant transformation. Remarkably, research in the p53 field has focused predominantly on these cell-autonomous mechanisms [2,4,5].

Yet, several studies over the last decade have highlighted a potential paracrine role for this versatile tumor suppressor. Apparently, p53 activation within a cell affects not only that cell but also its surroundings, by modulating the expression of genes that encode for secreted factors. In the context of tumors, such phenomena would pertain to the stromal component, namely the non-transformed cells of a variety of tissue origins that are usually a major part of any cancerous growth. Specifically, the activity of p53 within these cells could potentially impact upon the growth and viability of neighboring cancer cells. In addition, some reports indicate that p53 activation in a normal tissue could influence not only cells in the immediate neighborhood, but also distantly localized tumors, through endocrine mechanisms. Indeed, p53 induction was shown to induce a significant change in levels of a large number of secreted proteins, and possibly also post-translational modifications in many of them, forming the “p53-secretome” [6]. It can be speculated that some of these factors can affect only nearby cells, while others are stable enough to circulate through the vascular or lymphatic system and impact cancer cells in distant organs.

The cancer microenvironment includes stromal fibroblasts along with extracellular matrix secreted by these fibroblasts, adipocytes, cells belonging to the immune system, blood vessels, muscles and a number of additional cell types. Much of the data about p53 in stromal cells, which will be addressed below, stems from studies on fibroblasts, but p53 might be playing equally important roles also in other types of stromal cells.

In this review, we will focus on the paracrine and endocrine roles of stromal p53. We will summarize the current knowledge about the molecular mechanisms by which these effects might take place, and discuss their impact on the response of tumors to anti-cancer therapy. We will also address the clinical implications of these relatively new pathways, interconnecting tumors and stroma through the activity of p53.

2 Biological effects of stromal p53

2.1. Effects on tumor growth and cancer cell proliferation

To assess the effect of p53 status of the host upon tumor development, identical tumor cells were inoculated into either p53 knock-out mice or wt control mice. Tumor latency was found to be reduced in p53-null hosts relative to their normal controls [7]. Since the predominant type of non-transformed cell in a cancerous growth is usually fibroblasts [8], the authors examined the contribution of the status of p53 specifically in host fibroblasts. An experimental scheme that was repeatedly used to study the role of various fibroblasts in tumor progression is xenograft co-inoculation studies. Potentially tumorigenic tumor cells are inoculated into recipient mice, with or without accompanying fibroblasts. When tumor cells were co-inoculated with p53-null fibroblasts, tumor growth was accelerated relative to the same tumor cells co-inoculated with wild-type fibroblasts [7]. Furthermore, tumors formed with p53-null fibroblasts demonstrated a higher proliferation rate (based on Ki-67 staining), and less apoptosis (based on TUNEL staining) compared to tumors formed with wt fibroblasts. Although the distinction between fibroblasts and epithelial cells in this study was based on morphological criteria only, the enhanced proliferation and reduced apoptosis appeared to occur mostly in the epithelial component of those tumors. Overall, this study implies that

stromal fibroblast p53 inhibits proliferation and enhances apoptosis of xenografted epithelial cancer cells, thereby inhibiting tumor progression [7].

In another study, Komarova et al. treated cancer cells with conditioned medium collected from p53-null cells or from cells carrying wt p53 [9]. Using this system, they could demonstrate *in vitro* paracrine-p53-mediated growth inhibition. In this model, stress-induced p53 activation in the donor cells was required for the conditioned medium to have a cancer-inhibitory effect. It may be speculated that cell-cell contact or cell proximity is required for growth inhibition by basal levels of p53, while following more vigorous p53 activation, higher concentrations of secreted factors can carry this effect over a longer range.

2.2. Effects on angiogenesis

In a seminal study published in 1994, Dameron et al. demonstrated that when fibroblasts derived from Li-Fraumeni patients lose their single wt p53 allele in culture, angiogenic features emerge, implying an anti-angiogenic activity of wt p53 [10]. These researchers went further to demonstrate that this phenotypic switch was due to loss of p53-inducible expression of the anti-angiogenic secreted protein thrombospondin-1 (Tsp-1). Since then, numerous additional p53 target genes were reported that are likely to contribute to an anti-angiogenic effect (see section 3.2). Activation of tumor p53 was shown to reduce neoangiogenesis in a mouse model, even when only part of the tumor cells harbored wt p53 [11]. This suggests a p53-mediated anti-angiogenic influence of cells on their surroundings. Indeed, in a p53-null fibrosarcoma cell line, exogenous expression of wild type p53 did not affect proliferation *in vitro*, but induced tumor dormancy through reduced vascularization *in vivo* [12]. A study by Narendran et al. reported that leukemic bone-marrow stromal cells that harbor p53 mutations produce elevated levels of VEGF and support the growth of leukemic cells [13]. However, to the best of our knowledge, formal demonstration of an *in vivo* inhibitory effect of stromal p53 on tumor angiogenesis has not been reported yet.

2.3. Effects on metastasis

As discussed in this review, p53 affects in numerous ways the pattern of proteins secreted by stromal cells. Since some of those secreted proteins have been implicated in metastatic seeding, it is plausible that stromal p53 may also affect metastasis. However, this issue has so far been assessed only indirectly. In particular, Kang et al. reported that elevated expression of prosaposin by tumor cell lines reduces their metastatic potential, without affecting their proliferation rate [14]. Prosaposin is the precursor of saposin, a lipid hydrolase activator. Elevated expression of prosaposin by prostate cancer cells, orthotopically injected into mice, led to induction of p53 locally within the tumor stroma and also distantly, in the mouse lungs. Presumably secondary to this, Tsp-1 production was induced in stromal fibroblasts of the tumors as well as of the lungs. Furthermore, Tsp-1 induction in the fibroblasts of distant organs was required for the prevention of metastasis by prosaposin. This study joins several others that have highlighted a thought provoking novel process in tumor progression; namely, a localized cancerous growth can induce changes in distant organs. These changes can prepare the distant organs for the future seeding and growth of metastatic cells, thus preparing a “pre-metastatic niche” [15]. Intriguingly, the study of Kang et al. implicates stromal p53 in distant organs as a guardian that interferes with the preparation of such pre-metastatic niche by promoting the secretion of anti-angiogenic proteins, and possibly also other metastasis-inhibitory factors.

3 Molecular mechanisms underlying the effects of stromal p53

3.1 Mechanisms of proliferation inhibition

One of the most robust p53 transactivation targets is the gene encoding p21^{WAF1/CIP1}, a cyclin-dependent kinase inhibitor that is responsible for p53-mediated G1 cell cycle arrest [16]. Trimis et al. [17] examined the role of p21^{WAF1/CIP1} in the effect of stromal cells on tumor growth in a mouse model. Co-inoculation of p21^{WAF1/CIP1}-null fibroblasts together with cancer cells into SCID mice accelerated the rate of tumor growth relative to the same cancer cells co-inoculated with wt fibroblasts. Treating wt fibroblasts with a short inhibitory RNA targeting the p21^{WAF1/CIP1} gene prior to co-inoculation had a similar stimulatory effect on tumor growth. This raises the possibility that the inhibitory effect of stromal p53 on cancer growth may be mediated, at least partly, through p53-dependent induction of p21^{WAF1/CIP1}, although the underlying molecular mechanism remains to be elucidated. For instance, it can be speculated that a proliferative stromal component is required for the growth of cancer cells *in vivo*, possibly simply as a mechanical scaffold, and this proliferative capacity of the stromal cells is restricted by normal levels of p21^{WAF1/CIP1}. Interestingly, p21^{WAF1/CIP1} immunostaining in breast cancer stroma was enhanced in the vicinity of benign tumors, while in more advanced carcinomas fewer stromal cells were p21^{WAF1/CIP1}-positive [17]. The upregulation of p21^{WAF1/CIP1} in stromal cells adjacent to early stage tumors might be an indication of p53 induction by a tumor-originated factor. Advanced tumors might have acquired a mechanism to silence stromal p53, as reflected by the reduced p21^{WAF1/CIP1} immunostaining.

A number of other p53 transactivation targets may potentially mediate directly paracrine growth inhibition, and, when secreted by stromal cells, may suppress tumor cell proliferation. One such example is insulin-like growth factor binding protein-3 (IGF-BP3), which can inhibit mitogen signaling mediated by insulin-like growth factor-1 [18]. Early tumor cells are likely to be particularly susceptible to such inhibitory effects, since more advanced tumor cells tend to become increasingly less dependent on exogenous growth factors for proliferation and survival. Another pertinent example is PTGF- β , a p53 transactivation target gene product that inhibits tumor growth through the TGF- β pathway [19]. Komarova et al. identified several additional secreted p53-inducible proteins that possess growth inhibitory effects, including TGF- β 2, inhibin- β and a variety of serine-protease inhibitors [9]. The relative contribution of each of these molecules to stromal p53-mediated suppression of tumor growth remains unknown.

3.2 Molecular mechanisms relating stromal p53 to angiogenesis

Several transactivation targets of p53 encode potent antiangiogenic factors, including Tsp-1 [10], maspin [20] and BAI-1 [21]. In addition, p53 was shown to repress the transcription of genes encoding major angiogenic factors such as VEGF-1 [22] and bFGF [23]. While wt p53 inhibits transcription of the bFGF gene, cancer-associated mutant p53 can transactivate it [23]. Interestingly, a single nucleotide polymorphism found in 6% of the population creates a p53-responsive element within the *VEGFR-1* gene promoter [24]. VEGFR-1 seems to function mostly as a negative regulator of the VEGF pathway, positioning p53 again as an inhibitor of angiogenesis. An additional potential mode of inhibition of the VEGF pathway by p53 is through the Mdm2 protein, encoded by another pivotal p53-inducible gene. Mdm2 has been reported to promote the degradation of the transcription factor HIF-1 α , thereby reducing the angiogenic response to hypoxia [25]. Notably, the induction of TSP-1 and the degradation of HIF-1 α can be triggered already by endogenous, non-activated p53. Most, if not all, of these p53-dependent changes in angiogenesis regulators were demonstrated through the use of cancer cell lines, underscoring another incentive for cancer cells to lose their p53. However, in the *in vivo* setting, a substantial portion of angiogenesis regulatory proteins are secreted by the stromal

compartment, suggesting that stromal p53 may have a significant impact on tumor angiogenesis.

We reported previously that basal levels of p53 repress the production of the chemokine SDF-1/CXCL12 in embryonic fibroblasts of both human and mouse origin [26]. A similar effect can also be observed in fibroblasts derived from adult mice, and the repression of SDF-1 production in such cells can be further augmented pharmacologically by treatment with p53 activating drugs such as Nutlin (Fig. 1). Besides the involvement of SDF-1 in migration and invasion, as discussed below, SDF-1 is a major recruiter of endothelial progenitor cells that are believed to originate from the bone marrow and home to tumors, where they contribute to vasculogenesis [27]. This might be another mechanism whereby attenuated p53 in stromal cells enhances tumor vascular supply, fueled by SDF-1 upregulation.

3.3 Molecular mechanisms linking stromal p53 to prevention of metastasis

Metastasis formation is the most common cause of cancer-related death. It is the endpoint of a complex process, involving migration of tumor cells, invasion through blood vessel walls into the blood stream, attachment and survival in the recipient organ, culminating with proliferation and neovascularization of the new metastatic foci. Stromal p53 can be linked to many of these processes. Some of the molecular mechanisms mentioned above relating to proliferation, survival and angiogenesis are obviously relevant also to metastasis formation. In addition, stromal p53 may also have an impact on cancer cell migration and invasion, two biological processes closely linked to metastasis.

As mentioned above, SDF-1 expression was found to be transcriptionally downregulated by p53 in fibroblasts [26]. SDF-1 is a chemokine that can modulate migration and invasion of cancer cells that express the receptor for SDF-1, CXCR4. Such cells migrate and invade towards the source of SDF-1, and similarly towards conditioned medium produced by p53-null fibroblasts, a response that is abrogated by inhibition of the CXCR4 pathway in the responding tumor cells [26]. Conceivably, SDF-1 inhibition by functional p53 within normal stromal fibroblasts reduces this pro-metastatic signal, while in fibroblasts in which p53 has been incapacitated SDF-1 becomes upregulated, promoting cancer metastasis. Interestingly, Orimo et al. observed that CAFs derived from breast cancer patients promote tumor progression of co-inoculated cancer cells by secreting SDF-1 [27]. However, the activity status of p53 in the CAFs studied by Orimo et al. has not been reported.

Senescent fibroblasts were found to secrete a plethora of proteins, whose collective effect led to enhanced invasion and epithelial-to-mesenchymal transition (EMT) of cancer cells [28]. Loss of wt p53 in the senescent cells exacerbated this effect. Interleukin-6 (IL-6) and interleukin-8 (IL-8) were the dominant mediators of this influence. Of potential relevance, the IL-6 gene promoter can be repressed by wt p53 [29].

Matrix metalloproteinases (MMPs) regulate many processes related to invasion and metastasis. Degradation and remodeling of extracellular matrix (ECM) and release and activation of ECM-anchored growth factors are examples of MMP-modulated, metastasis-related effects. Wt p53 represses, and some p53 mutants activate MMP13 gene expression [30]. Moreover, wt p53 downregulates MMP1 [31,32], and upregulates MMP-2 [33]. While the combinatorial outcome of these influences is not easily evident, it most probably contributes to the effect of stromal p53 on cancer progression.

4. Effects of tumor cells on stromal p53

If indeed stromal p53 is a relevant inhibitor of epithelial tumor growth, it is expected that tumor cells that thrive in a microenvironment of cells with functional wt p53 would evolve

mechanisms to overcome this stromal p53-dependent inhibition. One possible mechanism might involve paracrine inhibition of stromal p53 by cancer cells, a process relying on the continuous presence of cancer cells and production of p53-inhibitory factors by them. Alternatively, stromal p53 function might be eliminated or attenuated by a process of selection that occurs in the course of tumor progression, if a significant advantage exists for stromal cells that lose their wt p53. In the latter case, reduced stromal p53 function is expected to be retained, at least for limited time periods, even if the stromal cells are placed in an environment that does not contain any cancer cells, e.g. by being transferred to tissue culture. Interestingly, evidence for both mechanisms has been presented.

Negative selection against stromal p53 during tumor progression was demonstrated very elegantly by Hill et al., studying co-evolution of the epithelial and stromal compartments in a mouse model of prostate cancer driven by a truncated SV40 large T antigen viral oncogene [34]. Early during tumor progression, these mice exhibit upregulation of p53 in the stromal fibroblasts adjacent to the hyperproliferative transformed prostate epithelium. Presumably, stromal p53 is induced by a paracrine signal emanating from the oncogene-stressed epithelial cells. Upon further progression of the tumor, in mice carrying a knockout of one p53 allele, loss of the remaining wt p53 allele became evident in the stroma, concurrently with enhanced abnormal proliferation of the stromal fibroblasts. The model that emerges from this study implicates a proliferative signal that is conveyed from early epithelial malignancies to the adjacent stroma. Stromal p53 is responding to this signal, activating a biological checkpoint that prevents anomalous proliferation of the stroma. Subsequent selection of stromal cells with defective p53 function then occurs, enabling the cancer cells to elicit a more effective stromal reaction and create a microenvironment that is more supportive of their proliferation and survival. Several reports of p53 aberrations in stromal fibroblasts and other stromal components of human tumors seem to support this model (see below).

Recent evidence from our laboratory supports a direct inhibitory affect of cancer cells on stromal p53 [35]. Specifically, conditioned medium collected from cultured lung cancer cells was found to attenuate the activation of fibroblast p53 by genotoxic stress. Of note, such inhibition was not exerted by cultured normal lung-derived epithelial cells, implying that the ability of cancer cells to suppress p53 activation is acquired as part of the malignant transformation process. Interestingly, cancer-associated fibroblasts (CAFs) responded with stronger p53 attenuation, compared to isogenic normal fibroblasts obtained from a non-cancerous region of the lungs of the same patients. These findings support the notion that inhibition of stromal p53 during tumor progression can be attained through convergent complementary processes, involving both a paracrine inhibitory signal emanating from the cancer cells and an inherent abnormality of the CAFs, presumably selected for in the microenvironment of the growing tumor (Fig. 2).

A recent study [36] suggests an unexpected way by which tumor cells might affect the p53 of stromal cells. Thus, stromal cells might undergo p53 inactivation by uptake of cancer cell-derived DNA. It was found that apoptotic bodies of transformed cells can be taken up by normal fibroblasts and endothelial cells. Stromal wt p53 prevented proliferation and replication of the foreign DNA. However, when the apoptotic bodies included DNA of SV40 large T antigen, the recipient stromal cells thrived and proliferated, possibly due to inhibition of their p53 by the exogenous SV40 large T antigen protein, which binds and inactivates p53. Uptake of tumor DNA by stromal cells was demonstrated also in mice bearing implanted tumors, even without any apoptosis-inducing treatments [36]. Interestingly, host endothelial cells were among the recipients of the foreign DNA. These observations raise the provocative possibility that cancer cells might induce angiogenesis by horizontal transfer of tumorigenic DNA to endothelial cells. Based on the tissue culture studies of Ehnfors et al, it might be expected that this mechanism would require the prior inactivation of the endothelial cell p53. Such inactivation might be

achieved directly by tumor DNA encoding p53-inhibitory proteins as in the model mentioned above, or more probably through other mechanisms, such as those implied by the studies described earlier [34,35]. Importantly, uptake of foreign DNA, particularly in broken form, is likely to activate stromal cell p53 via the DNA damage response pathway, imposing selective pressure for loss or attenuation of p53 function in the stroma.

5. Effect of stromal p53 on the response to anti-cancer agents

The study of Komarova et al. [9], mentioned above, also investigated the impact of p53 status of fibroblasts upon the response of co-cultured cancer cells to chemotherapy treatment. The cancer cells chosen for the study were resistant to chemotherapy owing to P-glycoprotein-mediated multi-drug resistance, leading to reduced intracellular drug accumulation. Nevertheless, these cancer cells died, probably through apoptosis, when grown on wt fibroblasts, a phenomenon not seen on a background of p53-null fibroblasts. Since these cancer cells are not affected by the chemotherapy itself, it is conceivable that their death must have been due to a p53-induced factor(s) produced by the co-cultured, co-treated fibroblasts. Indeed, conditioned medium of wt fibroblasts was found to cause growth inhibition of cancer cells, but only if collected after p53 activation by γ -irradiation or by other means. Interestingly, urine samples of irradiated wt mice also were capable of exerting a growth inhibitory effect on cancer cells, not seen with p53-null counterparts. Similarly to the *in vitro* studies involving conditioned medium, *in vivo* induction of p53 activation in the wt mice was required for their urine to exhibit a cancer growth-inhibitory influence [9]. A related study demonstrated that the growth of tumors implanted in mice was inhibited when a different organ of the same mouse, distant from the tumor, was γ -irradiated [37]. This phenomenon has been observed sporadically also in clinical practice and was termed “abscopal effect”. Camphausen et al. demonstrated the abscopal effect in their experimental model to be dependent on the wt p53 status of the mice. This is another example of a presumed p53-dependent secreted tumor-inhibitory factor that is activated by genotoxic treatment of the host tissues. In the reality of clinical practice, even the most targeted radiotherapy treatments, and obviously all systemically given chemotherapies, induce genotoxic stress in normal host cells. It may be speculated that part of the response of cancers to these treatments depends on the induction of p53 in normal tissues, including those of stromal derivation, and the resultant secretion of p53-dependent tumor-inhibitory factors.

The molecular and biological mechanisms underlying the response of cancer cells to the inhibitory factors induced by stromal p53 remain to be studied in more detail. In some cases, these factors might induce the cancer cells to undergo a growth arrest. However, considering the cell cycle-specific effects of some chemotherapeutic agents, stromal p53-induced growth arrest of cancer cells might render the latter more resistant to particular anti-cancer agents rather than more sensitive, contrary to the observations mentioned above. Indeed, Lafkas et al. found that when tumor cells were coinjected into mice together with wt fibroblasts, tumors grew more slowly but also responded less effectively to chemotherapy, when compared to similar tumors generated in the presence of p53-null fibroblasts [38]. Surprisingly, under *in vitro* conditions, conditioned medium of chemotherapy-treated wt fibroblasts enabled enhanced growth of cancer cells relative to conditioned medium derived from similarly treated p53-null fibroblasts. Of note, the wt fibroblasts underwent chemotherapy-induced senescence at a higher rate, leading to the conjecture that following p53-mediated senescence induction, wt fibroblasts secrete survival factors that render adjacent cancer cells resistant to chemotherapy [38].

A pronounced effect of stromal p53 status on the response of tumors to anti-cancer treatments was also demonstrated by Burdelya et al, using a different mouse tumor model [39]. Thus, these authors also found that p53-null stroma augments the tumor response to chemotherapy and radiotherapy. However, they invoked a different mechanistic explanation, namely that p53-null endothelial cells are more sensitive to these anti-cancer treatments than their wt

counterparts, and implicated an enhanced anti-angiogenic effect of these treatments in the p53-null stroma as underlying the more effective tumor growth inhibition.

A similar phenomenon was documented in a study of an anti-angiogenic schedule of chemotherapy [40]. Tumor-associated endothelial cells were found to undergo apoptotic death when treated by chemotherapy given at relatively short intervals, leading to death of the cancer cells. However, when the same experiment was carried out in p53-null mice, marked differences were noted. Initially, the tumors grew similarly to untreated tumors, presumably due to lack of p53-dependent growth arrest and apoptosis of the tumor endothelial cells. The following cycle of chemotherapy, when given to a wt host, merely led to a sustained inhibition of tumor growth. However, in the p53-null mice, it brought about massive endothelial cell apoptosis [40], similar to the *in vitro* observations of Burdelya et al. This was accompanied by an almost complete necrotic death of the tumors in the p53-null hosts. The apoptosis of the endothelial cells in the p53-null hosts, which is p53-independent by definition, was probably a result of sustaining irreparable genomic damage that was prevented in the wt endothelial cells by p53-dependent growth arrest [40]. It is noteworthy that an anti-apoptotic effect of p53 in fibroblasts has already been reported earlier [41,42], and might be more relevant when considering responses of stromal cells possessing a normal p53 pathway.

A somewhat different picture is provided by a study addressing the role of p53 in the response to TNP-470, an anti-angiogenic agent that is cytostatic for endothelial cells [43]. p53-dependent p21^{WAF1/CIP1} induction in endothelial cells was found to be required for TNP-470-mediated endothelial cell cycle arrest. It is conceivable that as a cytostatic anti-angiogenic treatment, which relies on the induction of growth arrest of endothelial cells, TNP-470 might require endothelial p53 for its antitumoral activity. In contrast, antiangiogenic treatments that induce apoptosis of the endothelial cells might be paradoxically rendered inefficient by the endothelial cell p53.

Taken together, the findings discussed above illustrate that while loss of stromal p53 function may often facilitate tumor growth and progression in the untreated host, it may actually sometimes render the tumor more sensitive to certain types of anti-cancer therapy. Moreover, they highlight the fact that the impact of stromal p53 on the tumor response to therapy is strongly dependent on the biological mechanism of action of the particular therapy agent. Better understanding of the pertinent effects of stromal p53 at the level of the whole organism is therefore important when any distinct type of anti-cancer treatment is being contemplated.

6. clinical relevance

6.1 p53 status in the tumor stroma of mouse cancer models

Several studies have assessed the status of p53 in the non-transformed cells, comprising the tumor stroma, in various mouse models of cancer. As mentioned earlier, Hill et al., investigating a prostate cancer model, found that stromal cells lose their wt p53 and even engage in neoplastic proliferation [34], presumably as a result of selection of p53-null stromal cells in the face of a p53-inducing signal from the neighboring transformed epithelial cells.

More recently, Dudley et al. reported that stromal cells expanded *in vitro* from several mouse tumor models display reduced p53 protein levels and activity [44]. p53 induction by genotoxic stress, as well as cell death following cytotoxic treatments, were attenuated in those CAFs. Unlike in the study of Hill et al., no structural alterations in the *p53* gene were found in the models assessed by Dudley et al, leaving unresolved the mechanism underlying the downregulation of p53 in the CAFs. Yet, given that the deficiency in p53 function could be observed even after extended growth of the CAFs *in vitro*, one must conclude that it is underpinned by genetic or epigenetic alterations that have occurred in those cells. While

epigenetic repression of the p53 gene itself remains an appealing potential mechanism, it is perhaps more likely that, at least in most cases, p53 functionality in CAFs is attenuated by stable alterations affecting directly the expression of other components of the p53 pathway. Interestingly, cultured human CAFs are more sensitive than normal fibroblasts to p53 inhibition by cancer cell conditioned medium, [35], in line with a possible stable modulation of the *p53* gene or pathway in those cells.

Together, the studies discussed above are consistent with the notion that stromal p53 affects tumor growth and progression, and in turn is modulated by cancer cell-derived factors. The precise molecular events responsible for modulation of p53 functional status in the stroma are probably dependent upon the type of initial offending oncogenic insults driving the transformation of the adjacent cancer cells, the tissue involved and the type of stromal cells recruited to the tumor, as well as on systemic factors. Further understanding of the molecular events governing those interactions is likely to be very rewarding.

6.2 p53 mutations in human cancer stroma

To validate the relevance of stromal p53 to human cancer, evaluating stromal cells taken from real human tumors is essential. An apparently simple question regards the presence or absence of p53 gene (TP53) mutations in such cells. Indeed, numerous reports have tried to answer this question. However, to this date the picture is far from clear, and major controversies still exist and are the focus of an ongoing debate. The first report of stromal p53 mutations dates back to the work of Wernert et al, who described the presence of mutant p53 in the stroma of colon and breast cancers [45]. Subsequently, a high prevalence of p53 mutations was described in breast cancer stroma [46], along with loss of heterozygosity (LOH) of the TP53 gene locus [47]. p53 mutations were found also in 2 out of 12 samples of leukemia bone marrow stromal cells [13]. Breast cancer-associated stromal p53 mutations were found to correlate with LOH of the ATM locus in those stromal cells. Since lack of either p53 or ATM leads to genomic instability, this is lending to speculate that one of these events may cause the other [47]. p53 mutations were reported to be more common in stromal cells of hereditary breast cancers compared to those of sporadic breast cancers [48], suggesting that germline BRCA gene abnormalities may predispose to stromal p53 mutation. Importantly, the existence of p53 mutations in stromal cells of sporadic breast cancers was found to correlate with lymph node metastasis [48]. Recently, Hasebe et al [49] reported that enhanced p53 immunohistochemical staining in breast cancer stromal cells correlates with nodal metastasis and worse prognosis. Although the *p53* gene was not sequenced in that study, enhanced p53 staining is often indicative of the presence of p53 mutations and excessive accumulation of mutant p53 protein. Although this correlation between increased staining and p53 mutations has been established primarily for tumor cells, it is likely to apply also to stromal p53. These findings suggest a link between stromal p53 mutations and increased metastasis, and raise the possibility that stromal p53 at the site of a primary tumor may restrict the invasive capacity of the tumor cells and their ability to disseminate away into the lymphatic system, or even that mutant p53 in the stromal cells might act through an oncogenic gain of function to actively promote the pro-metastatic behavior of the adjacent tumor cells.

The p53 pathway was investigated also in a set of cultured breast CAFs [50]. Lower basal levels of p53 and compromised activation of p53 by genotoxic stress was found in those stromal cells compared to counterpart normal fibroblasts. The sequence of the *p53* gene was not assessed in this report.

Yet, other investigators have seriously criticized the reports about p53 mutations in cancer-associated stromal cells, suggesting that they reflect artifacts stemming from the analysis of suboptimal amounts of poor quality, formalin-fixed DNA [51]. Specifically, employing stroma of fresh frozen breast cancer tissues, Campbell et al. could not detect any mutations in the

TP53 gene [52]. Furthermore, no LOH or gene copy number alterations were found when CAFs from breast and ovarian cancers were examined [53]. Thus, the existence of gene mutations in stromal fibroblasts in general, including mutations of the p53 gene, still remains a subject of substantial disagreement [51,54].

6.3 Clinical implications and future directions

Although many questions still remain unanswered, the importance of stromal p53 seems certain. The presence or absence of mutations in stromal p53 is under debate, and validation of reliable methods to answer this question in an unequivocal manner is urgently needed. If indeed validated, the prognostic implications of mutant stromal p53 need to be assessed more comprehensively.

Further understanding of the status of p53 in endothelial cells of human tumors is particularly required. According to the mouse tumor model studies cited above, downregulation of endothelial cell p53 might render them more sensitive to specific chemotherapy schedules or to other anti-angiogenic treatments. Again, validated methods for assessing both the mutational and functional status of stromal, endothelial p53 are required in order to examine this idea in the context of clinical practice. If aggressive cancer types evolve a mechanism to downregulate endothelial p53, this might constitute an “Achilles’ heel” and render these cells an attractive therapeutic target by virtue of their inactive p53.

Some cancer cells acquire the ability to inhibit p53 activity in additional types of stromal cells, possibly by a paracrine mechanism. Such inhibition likely contributes to the proliferation and/or survival of the cancer cells, providing them with a more supportive neighborhood before treatment. However, this may become a disadvantage to the tumor when the patient is exposed to anti-cancer treatments and enhance the cancer’s sensitivity to therapy. Identification of the p53-inhibitory factors secreted by cancer cells may enable their therapeutic manipulation: on the one hand, their inhibition might interfere with tumor growth and serve as a therapeutic modality in its own right, while on the other hand their augmentation might enable better guided use of conventional as well as novel anticancer agents in cases where stromal p53 is interfering with the efficacy of those agents.

Under at least some conditions where stromal p53 remains functional, it may play an important role in enhancing the response of tumors to chemotherapy and radiotherapy. However, this has so far been demonstrated only in mouse studies, and needs to be investigated in human cancer patients. If indeed stromal p53 activation is a component of the mechanism driving tumor regression following such treatments, stromal p53 might become a valid therapeutic target. While most current paradigms focus on the targeting of treatments specifically to the cancer cells within the tumor mass, strategies that target the supporting stroma are gaining growing recognition as an alternative and often complementary approach, as best illustrated for antiangiogenic therapy. In the case of p53, this can even be taken one step further: it can be speculated that it might sometimes be advantageous to activate wt p53 even in normal host tissues, not associated with the tumor mass. Such an approach might be even more relevant for tissues that are distant from the tumor, and thus not subject to stromal p53 modulating effects emanating from the cancer cells. For example, one may contemplate the possibility of targeting γ -radiation to non-tumorous, low-proliferating tissues, where the side effects and danger of carcinogenic mutations would be minimal. Obviously, much additional basic and pre-clinical research is required before such an approach can be considered for human cancer patients.

The role of stromal p53 in tissues that are potential future metastatic sites in cancer patients is especially intriguing. According to the study of Kang et al. [14], activation of stromal p53 in those tissues might prevent future metastasis. Such a mechanism is in keeping with the current methods aiming at preventing anticipated metastasis, which involve the administration of

adjuvant chemotherapy or radiotherapy to cancer patients following the complete surgical removal of the cancer. Such adjuvant treatments have been shown to reduce the chance of metastatic disease recurrence, presumably through eradication of sub-clinical microscopic metastatic deposits. It can be speculated that such treatments should be tailored to activate wt p53 in the tissues that are most prone to develop metastasis. It can be further speculated that the differences among individual patients in the success of the adjuvant treatments may be due in part to variations in the extent of host stromal p53 activation. It will be of great interest to test these speculations in the clinic.

7. Concluding remarks

A growing number of studies imply that stromal p53 has an inhibitory effect on cancer growth, proliferation, angiogenesis and metastasis formation. Importantly, stromal p53 may have an opposite effect on the response to cytotoxic or anti-angiogenic treatments, with wt p53 increasing therapy resistance and an abrogated stromal p53 sensitizing to it. Stromal p53 is probably downregulated in many tumor types, but affirmative assessment of the mechanism of this downregulation is essential in order to consider its therapeutic modulation. Validated methods for assessing the status of stromal p53 in human cancer patients are required. Hopefully, ways will be found to incorporate this knowledge towards optimizing the use of current therapy regimens as well as developing novel anti-cancer therapeutic approaches.

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Fig. 1. p53 represses SDF-1 expression in cultured adult lung fibroblasts

Lung fibroblasts were prepared from 4 months old wild type (WT) mice or p53 knockout (KO) mice, and maintained in culture for the minimal number of passages required to obtain enough cells for RNA analysis. Cells were either left untreated (control) or treated with the p53-activating drug Nutlin-3a (25μM) for 24 hours. RNA was extracted and subjected to qRT-PCR mRNA analysis of *SDF-1*, *p21*, and *HPRT* as an internal control. *p21* (right) and *SDF-1* (left) mRNA levels are shown after normalization for the *HPRT*. As expected, *p21* mRNA are high in the wt fibroblasts and are further elevated upon Nutlin treatment, while they are very low in the p53-null fibroblasts. In contrast, *SDF-1* mRNA expression is repressed in the wt cells relative to their p53-null counterparts, and is further reduced upon Nutlin treatment in the wild type but not p53-null fibroblasts, implying specific repression of *SDF-1* gene expression by wt p53.

Fig. 2. Proposed model for the association between tumor progression and altered p53 function in stromal cells

The cartoon depicts the proposed impact of stromal p53 on the secreted protein profile ("secretome") of stromal cells, exemplified by fibroblasts. Functional p53 in stromal fibroblasts increases their ability to produce and secrete tumor inhibitory factors, while suppressing the production of tumor promoting factors. Loss or attenuation of p53 function in fibroblasts reverses this pattern, leading to a shift towards an overall tumor-promoting secretome. It is further proposed that, in the course of tumor progression, p53 activity within cancer-associated fibroblasts (CAFs) becomes attenuated through several distinct cell-autonomous and non cell-autonomous mechanisms. This leads to changes in the secretome of CAFs relative to normal fibroblasts, creating a more supportive microenvironment for the cancer cells.