

# Chapter 6

## p53 Mutation in the Genesis of Metastasis

W.A. Yeudall

**Abstract** Development of metastatic cancer is a complex series of events that includes genesis of tumor-related vascular and lymphatic systems, enhanced cellular motility, and the capacity to invade and survive at distant sites, as well as evasion of host defences. The wild-type p53 protein plays key roles in controlling these facets of tumor progression, and loss of normal p53 function can be sufficient to predispose tumor cells to gain metastatic properties. In contrast, dominant p53 mutants that have gained oncogenic functions can actively drive metastasis through a variety of mechanisms. This chapter aims to highlight these processes.

**Keywords** Extracellular matrix • Motility • Epithelial-mesenchymal transition • G-protein • Chemokine • Transforming growth factor beta • microRNA

### Introduction

Invasion of the surrounding or underlying tissues is a crucial step in the progression to a malignant phenotype, and likely requires altered cellular interactions with the extracellular matrix (ECM) and enhanced motility. Development of metastatic disease is a late and often fatal process through which the tumor cells become established at a site distant to that of the primary lesion. This requires multiple biological steps, including development of capillary networks and/or lymphatic vessels adjacent to the tumor (angiogenesis or lymphangiogenesis), intravasation

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into these vessels, transport through the blood or lymphatic system, extravasation from the circulation, and survival as a secondary tumor deposit. In the case of epithelial neoplasms, this may be preceded by a phenotypic change in the tumor cells by means of epithelial-to-mesenchymal transition (EMT), although this remains somewhat controversial [1]. More recent work has highlighted the formation of a pre-metastatic niche in potential target organs as a result of mobilization and accumulation of bone-marrow derived cells which prepare the secondary site to accommodate tumor cells.

More than 100 years ago [2], in an effort to explain why some tumors metastasize preferentially to specific target organs, Paget proposed the “seed-and-soil” hypothesis, in which tumor cells with metastatic potential (the “seed”) would only metastasize to, and survive in, sites with which they had compatibility (the “soil”). In an excellent review article [3], the modern concepts of seed and soil are highlighted, which include tumor heterogeneity, selective metastasis of cells with pre-existing metastatic potential, and the consequences of the interactions between the microenvironment (metastatic niche) and the tumor cells. Current models suggest that subpopulations of cells exist within the heterogeneous primary tumor which have gained mutations that confer the propensity to metastasize and survive in particular organs, and that these mutations may be acquired early during tumorigenesis [4]. Two elegant studies highlight this using sub-lines of MDA-MB-231 breast cancer cells that metastasize predominantly either to lung or bone [5, 6]. Microarray-based comparison of gene expression in primary tumors and lung metastases revealed that the products of some genes were implicated in growth at the secondary site only, whereas others also played a role in primary tumor growth [6]. In the case of cells that were metastatic to bone, differentially-expressed genes encoded products involved in osteolysis and angiogenesis, amongst others [5]. However, the authors reported that the “bone metastasis gene signature” was additional to a previously-identified “poor prognosis gene signature” [7] that was present in the primary tumor, suggesting that additional biological functions over and above those required for primary tumorigenesis are needed to facilitate metastatic spread. Potentially, a subpopulation of cells within the primary tumor might harbor these metastasis-specific mutations, or they may be acquired as a result of further genetic insult of the primary lesion.

## **p53 Mutation and Development of Metastasis**

It is well accepted that wild-type p53 is a key regulator of cellular homeostasis, and that its loss through deletion or mutation underpins the development of many human malignancies by abrogating cell cycle checkpoints, cell death pathways and various other functions, some of which are pertinent to metastatic spread. One such mechanism involves regulation of neovascularization, a critical early step in metastasis, as outlined above. Some years ago, Van Meir and coworkers reported that expression of wild-type p53 in glioblastoma cells resulted in release of an angiogenesis inhibitor [8], while separate studies identified TSP-1, a potent inhibitor of angiogenesis, as a direct target of wild-type p53 [9], which impacts on survival [10]. Therefore, its

reduction in tumors with p53 loss-of-function would enhance the formation of a tumor blood supply. Wild-type p53 is now known to regulate angiogenesis through a number of mechanisms – by upregulating expression of angiogenesis inhibitors that include EphA2 [11–13] and BAI1 [14]. Conversely, wild-type p53 is reported to repress expression of proangiogenic molecules such as matrix metalloprotease (MMP)-1, hypoxia-inducible factor (HIF)-1 $\alpha$  [15], the HIF-1 $\alpha$  target vascular endothelial cell growth factor (VEGF)-A [16, 17], and cyclo-oxygenase (COX)-2 [18]. Other studies also indicate that some pro-angiogenic chemokines [19], including CXCL12, CXCL8 and CXCL5, are repressed by wild-type p53 [20, 21]. Indeed, in a cellular progression model of sarcomagenesis in which wild-type p53 was inactivated, Milyavsky et al. reported elevated expression of CXCL1 and CXCL8, amongst other genes, in the latter stages of tumor progression [22].

Wild-type p53 is also important in attenuating cellular migration and invasion. Of course, there is some overlap between genes involved in regulation of angiogenesis and cell motility. MMP-1 (collagenase IV) is a key enzyme required for degradation of extracellular matrix (ECM) as a component of the metastatic process, in addition to its role in angiogenesis. Other examples include the chemokines CXCL5, CXCL8 and CXCL12, which are repressed by wild-type p53 [20, 21] and which are key players in cell migration and metastasis [23–25]. Considerable insight into the role of p53 was provided by expression profiling studies conducted by Zhao and colleagues [26], who examined wild-type p53-dependent gene expression in a physiological setting. Amongst a cohort of targets that were either activated or repressed following induction of wild-type p53, they found activation of genes encoding  $\alpha$ 1 collagens type II and type VI, as well as structural proteins including actin and several keratins. Increased expression of plasminogen activator inhibitor-1 (PAI-1, SERPINE1), an inhibitor of the protease urokinase plasminogen activator (PLAU) was also reported. However, this protein may be involved in potentiation of the metastatic process as well as its inhibition, and its actual function may depend on the concentration in the microenvironment and the context in which it is expressed. For example, at physiological levels it acts to promote angiogenesis and cellular invasion, whereas elevated concentrations are inhibitory [27], and may act to aid cellular detachment [28] in an integrin-dependent manner [29]. Zhao et al. also found p53-dependent induction of endothelin-2 (EDN2), a modulator of vasoconstriction. Yet, EDN2 was demonstrated to function as a chemoattractant for macrophages [30] and may modulate the inflammatory infiltrate of tumors as well as enhance invasion [31]. Why this would be induced by a tumor suppressor protein such as p53 is, thus far, unclear, but may be unrelated to its role in tumor biology.

Studies in a mouse model system of hepatocellular carcinoma also provide further understanding of the role of wild-type p53 in suppression of metastasis. Delivery of a polyomavirus middle T antigen using the RCAS system into the livers of transgenic mice expressing the viral receptor (TVA) was found to result in formation of hepatic adenomas. However, when this was performed on a p53-null background, invasive and metastatic tumors developed, with differential expression of 105 genes between benign and malignant tumors [32]. These included insulin-like growth factor (IGF)-2, cathepsin E, and the chemokines CCL8 (MCP-2, SCYA8) and CCL5 (RANTES, SCYA5), all of which have recognized roles in metastatic spread of tumors.

Wild-type p53 also suppresses prometastatic activity through inhibition of small GTPase activation. Using mouse embryo fibroblasts from p53-null and p19<sup>ARF</sup>-null animals, Guo and colleagues reported changes in actin reorganization, accompanied by activation of phosphoinositide 3-kinase (PI3K) and Rac1 [33]. Further work by this group [34] indicated that p53 loss led to increased focal adhesion formation, and that ROCK activation only partially mimicked the RhoA phenotype. Promotion of an invasive phenotype by expression of active RhoA, Rac1, or Cdc42, but not an activated Ras mutant, was also observed in p53-null cells. However, subsequent studies by Xia and Land revealed that loss of p53 function combined with activated Ras resulted in enhanced cell motility [35]. Co-expression of mutant p53<sup>R175H</sup> and H-Ras<sup>G12V</sup> led to more profound migration in both wound-closure and transwell assays, with increased activation of RhoA, but not Rac1 or Cdc42. Similar GTP loading of RhoA was observed when endogenous wild-type p53 was repressed by shRNA, as well as in p53-null HCT116 cells, indicating the importance of loss of function. Moreover, wild-type p53 was shown to enhance activity of p190 RhoGAP, thus minimizing accumulation of the GTP-bound (active) form of RhoA.

## **p53 Gain-of-Function Mutants**

Over and above the undoubted importance of loss-of-function mutations in the development of metastatic disease, many common mutations in the p53 gene in human cancer result in expression of proteins with dominant transforming properties that actively drive tumor progression. These gain-of-function (GOF) mutants may endow the cells with many properties that impart growth or survival advantages, and may include functions pertinent to metastatic spread. Indeed, mouse models with knock-in GOF p53 alleles exhibit increased metastasis compared to p53-null animals [36–38]. GOF mutant p53 may also be associated with EMT, as mice expressing a mutant KRAS gene together with p53R172H were found to develop spindle cell carcinomas and frequent (>60 %) spread to secondary organs, including lungs and lymph nodes [38]. Nude mouse xenografts of fibroblasts expressing GOF p53 (H179L) were also shown to undergo metastatic spread, with deposits in lung and mediastinum from subcutaneous primary tumors [39].

## **Gain-of-Function Mechanisms**

Although there is debate over the mechanism through which GOF p53 mutants act, several models have been proposed. Some years ago, transcriptomic profiling provided clues to the aberrant functions of this class of proteins. Studies in lung cancer cells expressing specific p53 mutants revealed key differences (as well as some similarities) compared to the same cells expressing wild-type p53 [40, 41], and at least in some cases this appeared to be dependent upon the transactivation

properties of p53 as L22Q/W23S mutants (*i.e.*, in one of the transactivation domains) showed greatly reduced potency. Amongst the genes identified by this method, NF- $\kappa$ B2 (p100/p52) was upregulated by GOF p53 proteins [42], raising the possibility that transcription factors such as this may be activated by mutant p53, thereby leading to a secondary level of gene induction or repression that mediates the biological responses.

An important study by Weisz and colleagues investigated the effects of GOF p53 on the response of cancer cells to tumor necrosis factor (TNF)- $\alpha$  [43]. Whereas TNF- $\alpha$  is potentially cytotoxic, as it is able to induce an apoptotic response, it is also well-recognized that this cytokine can promote tumorigenesis through the activation of NF- $\kappa$ B on a background of inflammation [44, 45]. GOF p53 was shown to promote NF- $\kappa$ B activity in response to TNF- $\alpha$ , with increased nuclear translocation of p65, and to inhibit TNF- $\alpha$ -induced apoptosis, whereas cells lacking p53 or treated with siRNA showed increased cell death in the presence of ligand. Moreover, co-expression of mutant p53 and NF- $\kappa$ B was found in human tumors, further suggesting a functional relationship. Thus, GOF p53 may act as molecular switch that toggles the response to an otherwise cytotoxic factor through activation of NF- $\kappa$ B.

Another proposed mechanism to explain p53 gain-of-function mutation is through interaction with other members of the p53 family, such as p63 and p73. Whereas p73 may be of more importance in apoptosis, p63 isoforms have been linked to tumor progression and metastasis, although there is considerable controversy in the existing literature as to whether p63 proteins function as tumor suppressors or tumor promoters, which isoforms are responsible, and in which tissue types [46]. The presence of two promoters leads to expression of full-length TAp63 and  $\Delta$ Np63 which lacks the amino terminus. Each of these undergoes alternative splicing at the 3' end to generate  $\alpha$ ,  $\beta$  and  $\gamma$  forms and, potentially,  $\delta$  and  $\epsilon$ .  $\Delta$ Np63 is required for normal epithelial differentiation, and its absence is lethal due to deficient formation of the epidermis and associated structures.

Despite some apparent contradictory functions and activities reported for p63, emerging evidence seems to support the hypothesis that p63 acts as a suppressor of metastasis, and that the balance of expression between TA and  $\Delta$ N isoforms is important, with  $\Delta$ Np63 (or GOF mutant p53) being capable of oligotetramerizing with TAp63 and suppressing its anti-tumorigenic and anti-metastatic properties. Mice in which TAp63 is inactivated lose the Ras-dependent senescence response and show increased sarcomagenesis in the absence of p53 [47]. Consistent with this suppressive effect, gene knockdown of p63 in squamous carcinoma cell lines led to increased expression of a cohort of genes involved in invasion and metastasis that included  $\alpha$ 4 integrin, N-cadherin, tenascin C and two Wnt proteins, Wnt-4 and -5a, with a concomitant increase in cellular migration rate [48]. Moreover, mutant p53 has been shown to increase Rab-dependent recycling of the EGFR and  $\alpha$ 5 $\beta$ 1 integrin, inhibit the function of TAp63, and promote random migration, invasion and metastasis [49].

Maspin, also known as SERPINB5, is well-known for its function as an inhibitor of cell migration, invasion, metastasis and angiogenesis [50]. Although multiple factors contribute to altered maspin expression in human cancer, Kim et al.

identified TAp63 as an important activator of maspin expression in lung cells through binding to a p53 binding site in the maspin promoter [51]. These authors found a striking correlation between expression of maspin and p63 in lung cancers, with loss of both in adenocarcinomas and reduced expression of maspin in lung cancer metastases to lymph node. TAp63 was found to activate maspin expression in reporter gene assays, and maspin levels correlated inversely with invasion, implying that loss (or inactivation) of TAp63 during tumor progression results in enhanced capacity for metastatic spread. Consistent with this notion of p63-dependent suppression of invasion by maspin, studies in endometrial cancer cells showed that expression of the GOF p53 mutant R273H, but not a loss of function p53 mutant, inhibited maspin expression and promoted migration and invasion [52].

Studies by Adomo and colleagues showed co-operativity between transforming growth factor (TGF)- $\beta$  and GOF p53, in which GOF p53 and Smad2 enter into a ternary complex with p63 and repress its anti-metastatic activity, in part through modulation of five target genes: Cyclin G2, Sharp-1, Follistatin, ADAMTS9 and GPR87 [53]. They found that raising the level of p63 altered the balance of GOF p53 to p63, and suppressed lung colonization by tumor cells introduced *via* the mouse tail vein, further establishing p63 as a suppressor of metastasis. Moreover, TGF- $\beta$  treatment of cells expressing GOF p53 enhanced cell migration. The model proposed involves interaction of the  $\alpha$ -domain of p63 with the MH2 domain of Smad2, and binding of the transactivation domain of GOF p53 by the Smad2 MH1 domain. Thus, this may be specific for p63 $\alpha$  isoforms. Interestingly, tumor suppressive functions of TGF- $\beta$  through the repression of maspin expression and consequent inhibition of cell migration are dependent upon the presence of wild-type p53 [54].

A third mechanism to explain mutant p53 gain of function is direct recruitment, together with other transcription factors, onto the promoters of specific target genes. For example, it has been reported that GOF p53 interacts with NF-Y and p300, activating NF-Y targets [55]. A pertinent example related to invasion and metastasis is given by the elegant work of Fontemaggi et al. [56], in which they examined regulation of ID4 by GOF p53 – E2F1 complexes. They found that expression of R175H and R273H isoforms in H1299 cells led to elevated expression of ID4, whereas repression of mutant p53, but not wild-type p53, by siRNA resulted in lower ID4 levels. Subsequent analyses revealed that E2F1 was required for GOF p53-mediated activation of ID4 expression. This was shown to facilitate stabilization of mRNAs encoding the pro-angiogenic (and pro-metastatic) chemokines IL-8 (CXCL8) and Gro- $\alpha$  (CXCL1). Furthermore, co-expression of p53 and ID4 in breast tumors was found to correlate with increased microvessel density, a measure of angiogenesis. Enhanced recruitment of CBP and STAT onto the NF- $\kappa$ B2 promoter by GOF p53 mutants [57] also explains some of the earlier observations that this transcription factor is upregulated by mutant p53 [42]. Direct nucleation of mutant p53 onto the promoter of the receptor tyrosine kinase AXL has also been shown recently [58], but this appears to be independent of transactivation ability of the mutant p53, as L22Q/W23S mutants in one of the transactivation domains were still able to enhance AXL expression. Stimulation of AXL expression by GOF p53 was correlated with

enhanced motility of lung cancer cells, supporting a role for this axis in aspects of tumor metastasis.

## **p53 and the Chemokine Network**

It is now well accepted that NF- $\kappa$ B signaling is central to the chronic inflammatory response that promotes tumor progression and metastasis [59, 60]. The chemokine network is a complex system of ligands and receptors whose primary roles are in immune cell activation and their recruitment to inflammatory foci through production of chemokines at the inflammatory site, with establishment of a chemokine gradient towards which inflammatory cells expressing the cognate receptor migrate [61]. However, chemokines and their receptors are now firmly established as major players in tumorigenesis, angiogenesis and metastasis [23–25, 62–65]. For example, Muller et al. reported upregulation of CXCR4 and CCR7 receptors on the surface of breast cancer cells and demonstrated that this enabled homing of tumor cells to target organs expressing the ligands for these receptors [66]. Multiple studies have also reported elevated expression of pro-angiogenic chemokines such as CXCL1, CXCL2, CXCL3, CXCL5 and CXCL8 in a wide range of tumor types [67–72]. Thus, the roles of different chemokine-receptor interactions in tumor progression are varied and widespread.

As mentioned earlier, an interesting study by Moskovits et al. [20] reported that wild-type p53 represses CXCL12 expression, thereby reducing cell migration. Moreover, subsequent studies from our own laboratory [21], which focused on the contribution of GOF p53 to cell motility, highlighted a positive influence of these aberrant proteins on chemokine expression. Expression of several chemokines is regulated, at least in part, by NF- $\kappa$ B family transcription factors [73–83], consistent with a promoting role for inflammation in carcinogenesis, and it is clear that GOF p53 proteins activate a transcriptional response that includes NF- $\kappa$ B [41, 42]. Thus, it is perhaps no surprise that chemokines are upregulated by GOF p53. This is a clear gain of oncogenic function, as p53-null cells show higher levels of chemokine expression compared to cells expressing wild-type p53, yet substantial increases in chemokine expression occur when GOF p53 proteins are present [21]. Moreover, levels of CXCL5, CXCL8 and CXCL12 are increased differentially, depending upon the amino acid substitution present in p53, suggesting a degree of allele-specificity, and appear to depend upon elevated transcription. Further, these enhanced chemokine levels correlated with increased cellular motility, consistent with a role in invasion and metastasis. However, it is likely that deregulation of NF- $\kappa$ B activity is not the only mechanism responsible for enhancing chemokine expression. At least in the case of CXCL1 (Gro- $\alpha$ ) and CXCL8 (IL-8), enhanced mRNA stabilization through GOF p53 – E2F1 activation of ID4, as noted above, is crucial [56]. In addition, inactivation of p63 may also be a key mechanism, either directly or indirectly [84]. Thus, multiple biochemical mechanisms triggered by GOF p53 may cooperate to deregulate the chemokine network in cancer cells and enhance their progression to a metastatic phenotype.

## MicroRNA-Mediated Control of Metastasis

Another key mechanism that regulates processes involved in tumor cell metastasis is the action of microRNAs (miRNAs). These are small, non-coding RNAs present throughout the genome, which bind to target sequences in messenger RNAs (mRNAs), effecting their degradation or repressing their translation. They are generated in a step-wise process, which involves, first, expression of a pri-miRNA that contains a characteristic stem-loop structure. This entity is processed by an enzyme – Droscha – into a pre-miRNA, generally between 70 base pairs (bp) and 100 bp in length. Following export into the cytoplasm, the pre-miRNA is cleaved by Dicer to generate the mature miRNA consisting of two strands of 20–25 bp in length, one of which becomes incorporated into the RNA-induced silencing complex (RISC).

A number of miRNAs have been implicated in suppression of metastasis, including miR-31, miR126, miR-206, miR-335, miR-130, and the miR-200 family [85, 86]. Processing of these requires Dicer, which is transactivated by TAp63 [87]. Therefore, transdominant inhibition of p63 by GOF p53 represents yet another mode of action through which mutant p53 may promote tumor metastasis, as well as other wide-ranging effects on cellular biology [88, 89]. In addition, miR-200 species target the EMT-associated transcription factors ZEB1 and ZEB2 for degradation [90, 91]; therefore inhibition of miR-200 processing through loss of Dicer activity may explain how GOF p53 can promote EMT [92].

A recent study has provided further evidence for GOF p53 regulation of microRNA that leads to enhanced invasion in breast cancer [93]. Expression of either miR-155 or GOF p53 in p53-null cells led to increased invasion and EMT. Subsequent experiments indicated a direct role for p63 in transcriptional repression of miR-155, which was relieved by the presence of GOF p53. Interestingly, these authors identified the transcriptional repressor, ZNF652, as a target of miR-155. ZNF652 blocks expression of multiple genes involved in EMT, including vimentin, EGFR, TGF- $\beta$ , and TGF $\beta$ R2. Thus, inactivation of p63 by GOF p53 enables miR-155 to inactivate ZNF652, thereby promoting emergence of the mesenchymal phenotype that is characteristic of many invasive epithelial malignancies. Unsurprisingly, low levels of ZNF652 were found to correlate with breast tumor invasion in clinical samples.

## Conclusions

p53 mutation impacts metastatic processes on multiple levels. Loss of wild-type p53 function relieves repression of angiogenesis and enhances motility. However, dominant oncogenic p53 proteins actively drive metastasis by promoting angiogenesis through upregulating the expression of chemokines and other angiogenic factors in tumor cells. GOF p53 mutants enhance metastasis by switching on transcriptional programs that promote a more aggressive biological phenotype, and by interfering with the metastasis-suppressive functions of p63. Our emerging



understanding of microRNAs in control of angiogenesis and metastasis provides a further layer of complexity to p53 function. However, the central role of p53 in development of aggressive cancers may provide opportunities for targeted therapeutic approaches, either of p53 directly or one or more of its mediators, to improve clinical outcome.

## References

1. Tarin D, Thompson EW, Newgreen DF (2005) The fallacy of epithelial mesenchymal transition in neoplasia. *Cancer Res* 65:5996–6001
2. Paget S (1889) The distribution of secondary growths in cancer of the breast. *Lancet* 133:571–573
3. Fidler IJ (2002) Critical determinants of metastasis. *Semin Cancer Biol* 12:89–96
4. Bernards R, Weinberg RA (2002) A progression puzzle. *Nature* 418:823
5. Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordon-Cardo C, Guise TA, Massague J (2003) A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* 3:537–549
6. Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, Viale A, Olshen AB, Gerald WL, Massague J (2005) Genes that mediate breast cancer metastasis to lung. *Nature* 436:518–524
7. van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH (2002) Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 415:530–536
8. Van Meir EG, Polverini PJ, Chazin VR, Su Huang H-J, de Tribolet N, Cavenee WK (1994) Release of an inhibitor of angiogenesis upon induction of wild type p53 expression in glioblastoma cells. *Nat Genet* 8:171–176
9. Dameron K, Volpert O, Tainsky M, Bouck N (1994) Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science* 265:1582–1584
10. Lawler J, Miao WM, Duquette M, Bouck N, Bronson RT, Hynes RO (2001) Thrombospondin-1 gene expression affects survival and tumor spectrum of p53-deficient mice. *Am J Pathol* 159:1949–1956
11. Brantley DM, Cheng N, Thompson EJ, Lin Q, Brekken RA, Thorpe PE, Muraoka RS, Cerretti DP, Pozzi A, Jackson D, Lin C, Chen J (2002) Soluble Eph A receptors inhibit tumor angiogenesis and progression in vivo. *Oncogene* 21:7011–7026
12. Dohn M, Jiang J, Chen X (2001) Receptor tyrosine kinase EphA2 is regulated by p53-family proteins and induces apoptosis. *Oncogene* 20:6503–6515
13. Cheng N, Brantley DM, Liu H, Lin Q, Enriquez M, Gale N, Yancopoulos G, Cerretti DP, Daniel TO, Chen J (2002) Blockade of EphA receptor tyrosine kinase activation inhibits vascular endothelial cell growth factor-induced angiogenesis. *Mol Cancer Res* 1:2–11
14. Nishimori H, Shiratsuchi T, Urano T, Kimura Y, Kiyono K, Tatsumi K, Yoshida S, Ono M, Kuwano M, Nakamura Y, Tokino T (1997) A novel brain-specific p53-target gene, BAI1, containing thrombospondin type 1 repeats inhibits experimental angiogenesis. *Oncogene* 15:2145–2150
15. Ravi R, Mookerjee B, Bhujwala ZM, Sutter CH, Artemov D, Zeng Q, Dillehay LE, Madan A, Semenza GL, Bedi A (2000) Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1 $\alpha$ . *Genes Dev* 14:34–44
16. Mukhopadhyay D, Tsiokas L, Sukhatme VP (1995) Wild-type p53 and v-Src exert opposing influences on human vascular endothelial growth factor gene expression. *Cancer Res* 55:6161–6165
17. Pal S, Datta K, Mukhopadhyay D (2001) Central role of p53 on regulation of Vascular Permeability Factor/Vascular Endothelial Growth Factor (VPF/VEGF) expression in mammary carcinoma. *Cancer Res* 61:6952–6957

18. Subbaramaiah K, Altorki N, Chung WJ, Mestre JR, Sampat A, Dannenberg AJ (1999) Inhibition of cyclooxygenase-2 gene expression by p53. *J Biol Chem* 274:10911–10915
19. Strieter RM, Burdick MD, Gomperts BN, Belperio JA, Keane MP (2005) CXC chemokines in angiogenesis. *Cytokine Growth Factor Rev* 16:593–609
20. Moskovits N, Kalinkovich A, Bar J, Lapidot T, Oren M (2006) p53 attenuates cancer cell migration and invasion through repression of SDF-1/CXCL12 expression in stromal fibroblasts. *Cancer Res* 66:10671–10676
21. Yeudall WA, Vaughan CA, Miyazaki H, Ramamoorthy M, Choi MY, Chapman CG, Wang H, Black E, Bulysheva AA, Deb SP, Windle B, Deb S (2012) Gain-of-function mutant p53 upregulates CXC chemokines and enhances cell migration. *Carcinogenesis* 33:442–451
22. Milyavsky M, Tabach Y, Shats I, Erez N, Cohen Y, Tang X, Kalis M, Kogan I, Buganim Y, Goldfinger N, Ginsberg D, Harris CC, Domany E, Rotter V (2005) Transcriptional programs following genetic alterations in p53, INK4A, and H-Ras genes along defined stages of malignant transformation. *Cancer Res* 65:4530–4543
23. Yeudall WA, Miyazaki H (2007) Chemokines and squamous cancer of the head and neck: targets for therapeutic intervention? *Expert Rev Anticancer Ther* 7:351–360
24. Zlotnik A (2004) Chemokines in neoplastic progression. *Semin Cancer Biol* 14:181–185
25. Miyazaki H, Takabe K, Yeudall WA (2013) Chemokines, chemokine receptors and the gastrointestinal system. *World J Gastroenterol* 19:2847–2863
26. Zhao R, Gish K, Murphy M, Yin Y, Notterman D, Hoffman WH, Tom E, Mack DH, Levine AJ (2000) Analysis of p53-regulated gene expression patterns using oligonucleotide arrays. *Genes Dev* 14:981–993
27. Bajou K, Maillard C, Jost M, Lijnen RH, Gils A, Declerck P, Carmeliet P, Foidart JM, Noel A (2004) Host-derived plasminogen activator inhibitor-1 (PAI-1) concentration is critical for in vivo tumoral angiogenesis and growth. *Oncogene* 23:6986–6990
28. Czekay RP, Aertgeerts K, Curriden SA, Loskutoff DJ (2003) Plasminogen activator inhibitor-1 detaches cells from extracellular matrices by inactivating integrins. *J Cell Biol* 160:781–791
29. Czekay RP, Loskutoff DJ (2009) Plasminogen activator inhibitors regulate cell adhesion through a uPAR-dependent mechanism. *J Cell Physiol* 220:655–663
30. Grimshaw MJ, Wilson JL, Balkwill FR (2002) Endothelin-2 is a macrophage chemoattractant: implications for macrophage distribution in tumors. *Eur J Immunol* 32:2393–2400
31. Grimshaw MJ, Hagemann T, Ayhan A, Gillett CE, Binder C, Balkwill FR (2004) A role for endothelin-2 and its receptors in breast tumor cell invasion. *Cancer Res* 64:2461–2468
32. Lewis BC, Klimstra DS, Socci ND, Xu S, Koutcher JA, Varmus HE (2005) The absence of p53 promotes metastasis in a novel somatic mouse model for hepatocellular carcinoma. *Mol Cell Biol* 25:1228–1237
33. Guo F, Gao Y, Wang L, Zheng Y (2003) p19Arf-p53 tumor suppressor pathway regulates cell motility by suppression of phosphoinositide 3-Kinase and Rac1 GTPase Activities. *J Biol Chem* 278:14414–14419
34. Guo F, Zheng Y (2004) Rho family GTPases cooperate with p53 deletion to promote primary mouse embryonic fibroblast cell invasion. *Oncogene* 23:5577–5585
35. Xia M, Land H (2007) Tumor suppressor p53 restricts Ras stimulation of RhoA and cancer cell motility. *Nat Struct Mol Biol* 14:215–223
36. Lang GA, Iwakuma T, Suh YA, Liu G, Rao VA, Parant JM, Valentin-Vega YA, Terzian T, Caldwell LC, Strong LC, El-Naggar AK, Lozano G (2004) Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. *Cell* 119:861–872
37. Olive KP, Tuveson DA, Ruhe ZC, Yin B, Willis NA, Bronson RT, Crowley D, Jacks T (2004) Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. *Cell* 119:847–860
38. Caulin C, Nguyen T, Lang GA, Goepfert TM, Brinkley BR, Cai WW, Lozano G, Roop DR (2007) An inducible mouse model for skin cancer reveals distinct roles for gain- and loss-of-function p53 mutations. *J Clin Invest* 117:1893–1901
39. Cardinali M, Kratochvil FJ, Ensley JF, Robbins KC, Yeudall WA (1997) Functional characterization in vivo of mutant p53 molecules derived from squamous cell carcinomas of the head and neck. *Mol Carcinog* 18:78–88

40. Scian MJ, Stagliano KE, Deb D, Ellis MA, Carchman EH, Das A, Valerie K, Deb SP, Deb S (2004) Tumor-derived p53 mutants induce oncogenesis by transactivating growth-promoting genes. *Oncogene* 23:4430–4443
41. Scian MJ, Stagliano KE, Ellis MA, Hassan S, Bowman M, Miles MF, Deb SP, Deb S (2004) Modulation of gene expression by tumor-derived p53 mutants. *Cancer Res* 64:7447–7454
42. Scian MJ, Stagliano KE, Anderson MA, Hassan S, Bowman M, Miles MF, Deb SP, Deb S (2005) Tumor-derived p53 mutants induce NF-kappaB2 gene expression. *Mol Cell Biol* 25:10097–10110
43. Weisz L, Damalas A, Lontos M, Karakaidos P, Fontemaggi G, Maor-Aloni R, Kalis M, Levrero M, Strano S, Gorgoulis VG, Rotter V, Blandino G, Oren M (2007) Mutant p53 enhances nuclear factor kappaB activation by tumor necrosis factor alpha in cancer cells. *Cancer Res* 67:2396–2401
44. Luo JL, Maeda S, Hsu LC, Yagita H, Karin M (2004) Inhibition of NF-kappaB in cancer cells converts inflammation-induced tumor growth mediated by TNFalpha to TRAIL-mediated tumor regression. *Cancer Cell* 6:297–305
45. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y (2004) NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature* 431:461–466
46. Melino G (2011) p63 is a suppressor of tumorigenesis and metastasis interacting with mutant p53. *Cell Death Differ* 18:1487–1499
47. Guo X, Keyes WM, Papazoglu C, Zuber J, Li W, Lowe SW, Vogel H, Mills AA (2009) TAp63 induces senescence and suppresses tumorigenesis in vivo. *Nat Cell Biol* 11:1451–1457
48. Barbieri CE, Tang LJ, Brown KA, Pietenpol JA (2006) Loss of p63 leads to increased cell migration and up-regulation of genes involved in invasion and metastasis. *Cancer Res* 66:7589–7597
49. Muller PA, Caswell PT, Doyle B, Iwanicki MP, Tan EH, Karim S, Lukashchuk N, Gillespie DA, Ludwig RL, Gosselin P, Cromer A, Brugge JS, Sansom OJ, Norman JC, Vousden KH (2009) Mutant p53 drives invasion by promoting integrin recycling. *Cell* 139:1327–1341
50. Zhang M (2004) Multiple functions of maspin in tumor progression and mouse development. *Front Biosci* 9:2218–2226
51. Kim S, Han J, Kim J, Park C (2004) Maspin expression is transactivated by p63 and is critical for the modulation of lung cancer progression. *Cancer Res* 64:6900–6905
52. Dong P, Tada M, Hamada J-I, Nakamura A, Moriuchi T, Sakuragi N (2007) p53 dominant-negative mutant R273H promotes invasion and migration of human endometrial cancer HHUA cells. *Clin Exp Metastasis* 24:471–483
53. Adorno M, Cordenonsi M, Montagner M, Dupont S, Wong C, Hann B, Solari A, Bobisse S, Rondina MB, Guzzardo V, Parenti AR, Rosato A, Bicciato S, Balmain A, Piccolo S (2009) A mutant-p53/Smad complex opposes p63 to empower TGF[beta]-induced metastasis. *Cell* 137:87–98
54. Wang SE, Narasanna A, Whitell CW, Wu FY, Friedman DB, Arteaga CL (2007) Convergence of p53 and Transforming Growth Factor beta (TGFbeta) signaling on activating expression of the tumor suppressor gene maspin in mammary epithelial cells. *J Biol Chem* 282:5661–5669
55. Di Agostino S, Strano S, Emiliozzi V, Zerbini V, Mottolese M, Sacchi A, Blandino G, Piaggio G (2006) Gain of function of mutant p53: the mutant p53/NF-Y protein complex reveals an aberrant transcriptional mechanism of cell cycle regulation. *Cancer Cell* 10:191–202
56. Fontemaggi G, Dell'Orso S, Trisciuglio D, Shay T, Melucci E, Fazi F, Terrenato I, Mottolese M, Muti P, Domany E, Del Bufalo D, Strano S, Blandino G (2009) The execution of the transcriptional axis mutant p53, E2F1 and ID4 promotes tumor neo-angiogenesis. *Nat Struct Mol Biol* 16:1086–1093
57. Vaughan CA, Singh S, Windle B, Sankala HM, Graves PR, Andrew Yeudall W, Deb SP, Deb S (2012) p53 mutants induce transcription of NF-kappaB2 in H1299 cells through CBP and STAT binding on the NF-kappaB2 promoter and gain of function activity. *Arch Biochem Biophys* 518:79–88

58. Vaughan CA, Singh S, Windle B, Yeudall WA, Frum R, Grossman SR, Deb SP, Deb S (2012) Gain-of-function activity of mutant p53 in lung cancer through up-regulation of receptor protein tyrosine kinase Ax1. *Genes Cancer* 3:491–502
59. Karin M, Greten FR (2005) NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol* 5:749–759
60. Karin M, Cao Y, Greten FR, Li ZW (2002) NF-kappaB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer* 2:301–310
61. Locati MDM, Murphy MDPM (1999) Chemokines and chemokine receptors: biology and clinical relevance in inflammation and AIDS1. *Annu Rev Med* 50:425–440
62. Balkwill F (2004) Cancer and the chemokine network. *Nat Rev Cancer* 4:540–550
63. Balkwill F, Charles KA, Mantovani A (2005) Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* 7:211–217
64. Strieter RM, Belperio JA, Phillips RJ, Keane MP (2004) CXC chemokines in angiogenesis of cancer. *Semin Cancer Biol* 14:195–200
65. Strieter RM, Burdick MD, Mestas J, Gomperts B, Keane MP, Belperio JA (2006) Cancer CXC chemokine networks and tumour angiogenesis. *Eur J Cancer* 42:768–778
66. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A (2001) Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410:50–56
67. Balentien E, Mufson BE, Shattuck RL, Derynck R, Richmond A (1991) Effects of MGSA/GRO alpha on melanocyte transformation. *Oncogene* 6:1115–1124
68. Dhawan P, Richmond A (2002) Role of CXCL1 in tumorigenesis of melanoma. *J Leukoc Biol* 72:9–18
69. Keane MP, Belperio JA, Xue YY, Burdick MD, Strieter RM (2004) Depletion of CXCR2 inhibits tumor growth and angiogenesis in a murine model of lung cancer. *J Immunol* 172:2853–2860
70. Kryczek I, Lange A, Mottram P, Alvarez X, Cheng P, Hogan M, Moons L, Wei S, Zou L, Machelon V, Emilie D, Terrassa M, Lackner A, Curiel TJ, Carmeliet P, Zou W (2005) CXCL12 and vascular endothelial growth factor synergistically induce neoangiogenesis in human ovarian cancers. *Cancer Res* 65:465–472
71. Strieter RM, Belperio JA, Burdick MD, Sharma S, Dubinett SM, Keane MP (2004) CXC chemokines: angiogenesis, immunoangiostasis, and metastases in lung cancer. *Ann N Y Acad Sci* 1028:351–360
72. Miyazaki H, Patel V, Wang H, Edmunds RK, Gutkind JS, Yeudall WA (2006) Downregulation of CXCL5 inhibits squamous carcinogenesis. *Cancer Res* 66:4279–4284
73. Kunsch C, Rosen CA (1993) NF-kappa B subunit-specific regulation of the interleukin-8 promoter. *Mol Cell Biol* 13:6137–6146
74. Kunsch C, Lang RK, Rosen CA, Shannon MF (1994) Synergistic transcriptional activation of the IL-8 gene by NF-kappa B p65 (RelA) and NF-IL-6. *J Immunol* 153:153–164
75. Yang J, Richmond A (2001) Constitutive IkappaB kinase activity correlates with nuclear factor-kappaB activation in human melanoma cells. *Cancer Res* 61:4901–4909
76. Wang D, Richmond A (2001) Nuclear factor-kappa B activation by the CXC chemokine melanoma growth-stimulatory activity/growth-regulated protein involves the MEKK1/p38 mitogen-activated protein kinase pathway. *J Biol Chem* 276:3650–3659
77. Chandrasekar B, Mummidi S, Perla RP, Bysani S, Dulin NO, Liu F, Melby PC (2003) Fractalkine (CX3CL1) stimulated by nuclear factor kappaB (NF-kappaB)-dependent inflammatory signals induces aortic smooth muscle cell proliferation through an autocrine pathway. *Biochem J* 373:547–558
78. Dhawan P, Richmond A (2002) A novel NF-kappa B-inducing kinase-MAPK signaling pathway up-regulates NF-kappa B activity in melanoma cells. *J Biol Chem* 277:7920–7928
79. Richmond A (2002) NF-[kappa]B, chemokine gene transcription and tumour growth. *Nat Rev Immunol* 2:664–674
80. Rehman AO, Wang C-Y (2008) SDF-1{alpha} promotes invasion of head and neck squamous cell carcinoma by activating NF-{kappa}B. *J Biol Chem* 283:19888–19894

81. Furuya M, Suyama T, Usui H, Kasuya Y, Nishiyama M, Tanaka N, Ishiwata I, Nagai Y, Shozu M, Kimura S (2007) Up-regulation of CXC chemokines and their receptors: implications for proinflammatory microenvironments of ovarian carcinomas and endometriosis. *Hum Pathol* 38:1676–1687
82. Maroni P, Bendinelli P, Matteucci E, Desiderio MA (2007) HGF induces CXCR4 and CXCL12-mediated tumor invasion through Ets1 and NF-kappaB. *Carcinogenesis* 28:267–279
83. Sun Q, Matta H, Lu G, Chaudhary PM (2006) Induction of IL-8 expression by human herpesvirus 8 encoded vFLIP K13 *via* NF-kappaB activation. *Oncogene* 25:2717–2726
84. Yang X, Lu H, Yan B, Romano R-A, Bian Y, Friedman J, Duggal P, Allen C, Chuang R, Ehsanian R, Si H, Sinha S, Van Waes C, Chen Z (2011) DeltaNp63 versatily regulates a broad NF-kappaB gene program and promotes squamous epithelial proliferation, migration, and inflammation. *Cancer Res* 71:3688–3700
85. Valastyan S, Reinhardt F, Benaich N, Calogrias D, Szasz AM, Wang ZC, Brock JE, Richardson AL, Weinberg RA (2009) A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. *Cell* 137:1032–1046
86. Valastyan S, Weinberg RA (2010) Metastasis suppression: a role of the Dice(r). *Genome Biol* 11:141
87. Su X, Chakravarti D, Cho MS, Liu L, Gi YJ, Lin YL, Leung ML, El-Naggar A, Creighton CJ, Suraokar MB, Wistuba I, Flores ER (2010) TAp63 suppresses metastasis through coordinate regulation of Dicer and miRNAs. *Nature* 467:986–990
88. Tucci P, Agostini M, Grespi F, Markert EK, Terrinoni A, Vousden KH, Muller PAJ, Dötsch V, Kehroesser S, Sayan BS, Giaccone G, Lowe SW, Takahashi N, Vandenabeele P, Knight RA, Levine AJ, Melino G (2012) Loss of p63 and its microRNA-205 target results in enhanced cell migration and metastasis in prostate cancer. *Proc Natl Acad Sci U S A* 109:15312–15317
89. Tran MN, Choi W, Wszolek MF, Navai N, Lee ILC, Nitti G, Wen S, Flores ER, Siefker-Radtke A, Czerniak B, Dinney C, Barton M, McConkey DJ (2013) The p63 protein isoform  $\Delta Np63\alpha$  inhibits epithelial-mesenchymal transition in human bladder cancer cells: role of MIR-205. *J Biol Chem* 288:3275–3288
90. Park S-M, Gaur AB, Lengyel E, Peter ME (2008) The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. *Genes Dev* 22:894–907
91. Korpai M, Lee ES, Hu G, Kang Y (2008) The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. *J Biol Chem* 283:14910–14914
92. Ohashi S, Natsuzaka M, Wong GS, Michaylira CZ, Grugan KD, Stairs DB, Kalabis J, Vega ME, Kalman RA, Nakagawa M, Klein-Szanto AJ, Herlyn M, Diehl JA, Rustgi AK, Nakagawa H (2010) Epidermal growth factor receptor and mutant p53 expand an esophageal cellular subpopulation capable of epithelial-to-mesenchymal transition through ZEB transcription factors. *Cancer Res* 70:4174–4184
93. Neilsen PM, Noll JE, Mattiske S, Bracken CP, Gregory PA, Schulz RB, Lim SP, Kumar R, Suetani RJ, Goodall GJ, Callen DF (2013) Mutant p53 drives invasion in breast tumors through up-regulation of miR-155. *Oncogene* 32:2992–3000