Genetics and Molecular Biology of Mesothelioma

9

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9.1 Apoptosis as a Tumor Suppressor Mechanism

Mesothelioma remains an incurable cancer due to the ineffectiveness of conventional cytotoxic chemotherapy. This is reflected in the preponderance of mostly negative phase II clinical trials over the last 30 years [32]. Resistance to apoptosis is a hallmark of cancer in general [48], accounts for multidrug resistance [58], and is a signature of mesothelioma [31]. During tumorigenesis, it is now understood that as in common with other solid cancers, somatic genetic alteration is a frequent event predisposing to apoptosis resistance. These changes include the activation of oncogenic cell survival pathways, and the inactivation of tumor suppressors.

This chapter will focus on how apoptosis susceptibility in mesothelioma is, in general, inhibited by the acquisition of multiple somatic alterations in oncogenic and tumor suppressor protein expression. Growing knowledge of these key genetic changes and their requirement for sustaining the malignant mesothelioma phenotype provide insights into potential vulnerabilities that may be successfully exploited using new therapeutic strategies. I will first of all, summarize our understanding of how the core death machinery is altered in mesothelioma (summarized in Fig. 9.1). This will be followed by a summary of the most frequent genetic alterations driving oncogenic pathways or leading to dysfunction of tumor suppressors (summarized in Fig. 9.2). Translational research opportunities arising from this knowledge of mesothelioma pathobiology will then be highlighted.

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9.2 Key Alterations in the Core Apoptosis Signaling in Mesothelioma

9.2.1 Regulation of the Intrinsic (Mitochondrial) Apoptosis Pathway in Mesothelioma

The BCL-2 family of proteins constitutes the pivotal molecular regulators of the core cell death machinery. This family is subdivided into proapoptotic and antiapoptotic proteins. BCL-2, the prototypical member of the BCL-2 family

A. Tannapfel (ed.), *Malignant Mesothelioma*, Recent Results in Cancer Research 189,
DOI: 10.1007/978-3-642-10862-4_9, © Springer-Verlag Berlin Heidelberg 2011

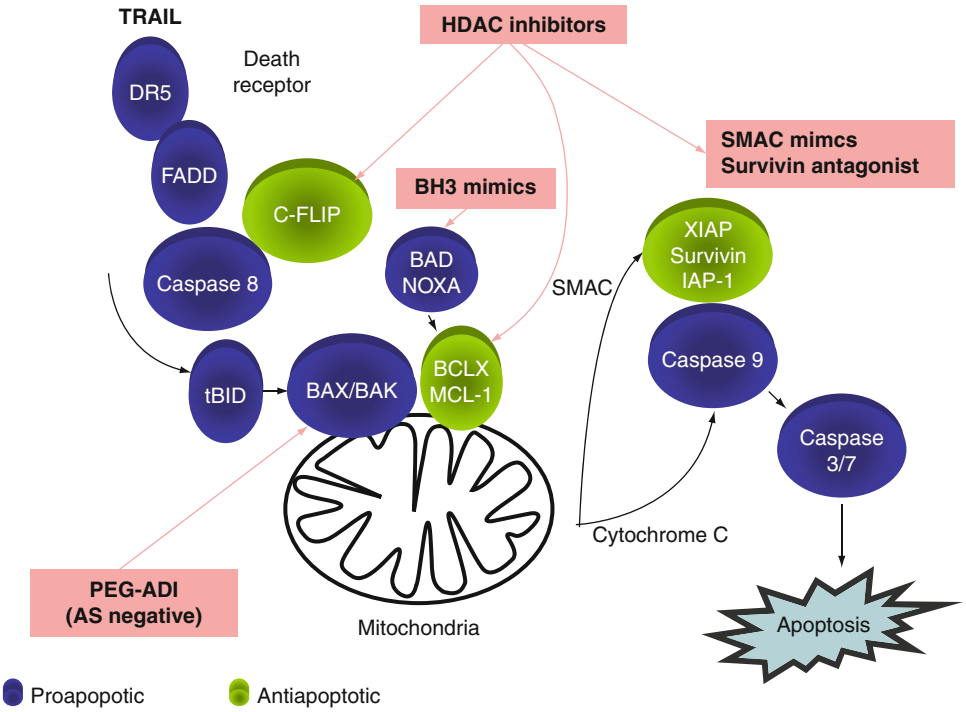

Fig. 9.1 Altered regulation of the core apoptosis pathway in mesothelioma. Upregulated antiapoptotic proteins are highlighted (*green*)

was identified as a proto-oncogene associated with the t(14;18) translocation in follicular lymphoma [129]. The antiapoptotic protein subgroup now includes five additional proteins, MCL-1, BCL-X, BCL-W, A1 and BCL-B. Prosurvival BCL-2 family proteins regulate apoptosis at the level of the mitochondrial and endoplasmic reticulum outer membranes. The canonical cell death pathway involves mitochondria; organelles responsible for generating ATP, the cell's energy currency, through oxidative phosphorylation.

Prosurvival BCL-2 family proteins function to block a critical death switch which is responsible for making the all-or-none decision to commit a cell irreversibly to death [4,24]. This switch is the permeabilization of the outer mitochondrial membrane, induced by oligomerization and pore formation by the tumor suppressors and multidomain proapoptotic proteins BAK and BAX [99,115,146,147]. Mitochondrial outer membrane permeabilization or MOMP is a rapid, kinetically invariant event that results in the release several proteins from the mitochondria into the cytosol. These proteins include cytochrome C [77], SMAC [27], OMI/HtrA2 [82] and apoptosis-inducing factor [122]. Cytochrome C in conjunction with APAF-1 [149] and dATP, triggers the activation of a family of zymogens called caspases, which cooperate in mediating cellular demolition by cleaving hundreds of substrates. Bax and Bak are genetically redundant tumor suppressors [140]; prosurvival BCL-2 proteins heterodimerize to prevent BAX and BAK activation, functioning as a rheostat that is dependent on the ratio of pro- to antiapoptotic proteins.

In common with other tumor suppressors, BAX deficiency has been identified in primary malignancies [84]. However, low bcl-2/bax ratio has been reported in mesothelioma cells despite their apoptosis resistance, implicating a mechanism other than BCL-2 in regulating apoptosis. In vivo, MCL-1 is more commonly

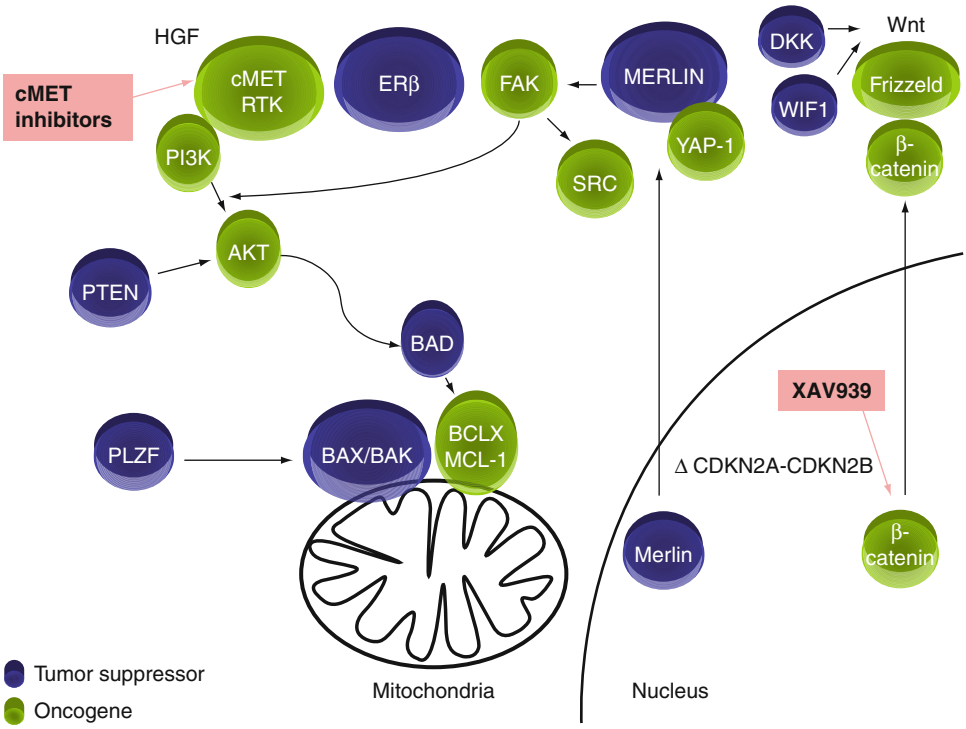

Fig. 9.2 Key proteins involved in survival pathway signaling in mesothelioma (*green*). Tumor suppressors are shown in *blue*

expressed whereas BCL-2 expression is less frequent [96,119]. BAX and BAK require a subset of proapoptotic BCL-2 family proteins for activation which share homology in a death-inducing BCL-2 homology 3 (BH3) domain, but do not contain other BH domains. Two such BH3 domain-only proteins, BID [136] and BIM [95] can directly induce the oligomerization and activation of BAX. Interestingly, in mesothelioma, loss of expression of BH3-only proteins has been reported in vivo, namely, BID (37%) and BIM (18%). In addition, loss of BAX expression has been reported in one series in 42% of primary mesotheliomas [96].

Prosurvival BCL-2 family proteins are inhibited by a subset of BH3-only proteins, which are incapable of direct BAX/BAK activation, but bind directly to prosurvival counterparts. These so-called dissociator BH3-only proteins reflect a growing family and include BAD, NOXA, PUMA, BMF, BIK and HRK. Because dissociator BH3-only proteins are endogenous prosurvival BCL-2 family inhibitors, they represent a prototype for small molecule drug development, most notably ABT737 [76,98]. BH3 mimetics are a promising class of mitochondria targeted therapy with potential for treating mesothelioma. This is suggested by studies in which silencing BCl-2 and BCL-XL was sufficient to induce apoptosis and chemosensitization [52]. However, target specificity is likely to be important for therapeutic efficacy. MCL-1 is highly expressed in mesothelioma and is one of the most commonly amplified oncogenes in human cancer [9]. It is also a resistance biomarker for ABT737 [68,132]. Nevertheless, other prosurvival BCL-2 family targeted agents such as obatoclax [92] are currently in clinical development, and may exhibit efficacy in mesothelioma.

9.2.2 Extrinsic Apoptosis Pathway Regulation in Mesothelioma

Apoptosis can be efficiently induced in mesothelioma cell lines by ligation of cell surface death receptors. Activation of death receptors by their ligands (which include TNF and FAS) leads to recruitment of FADD through a conserved death domain [22], followed by recruitment of caspase 8 [10] activating complex known as the death-inducing signaling complex or DISC [127]. Caspase 8 cleaves BID, leading to activation of BAX/BAK, mitochondrial apoptosis, and therefore signal amplification [78]. Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) or TRAIL receptor agonists are currently in clinical development but have yet to be evaluated in mesothelioma. Upon interaction, its receptors (TRAIL R1 or R2) can induce apoptosis in vitro. TRAIL also synergizes with DNA damage induced by etoposide in a manner that requires c-jun N terminal kinase [135].

FLIP is an inhibitor of TRAIL-induced apoptosis and is recruited to the DISC [90], where it inhibits caspase 8 recruitment and activation. Mesothelioma cells overexpress FLIP resulting in inhibition of death receptor-induced apoptosis [112]. Silencing of FLIP in mesothelioma and other cancer models re-establishes sensitivity to TRAIL [112,141]. Novel, clinically applicable approaches for downregulating FLIP in the clinical setting will be highlighted later in this chapter.

9.2.3 Inhibitors of Apoptosis in Mesothelioma

Inhibitors of apoptosis (IAPs) comprise a family of structurally related proteins, which share a common 70 amino acid baculovirus IAP (BIR) repeat. IAPs interact with and inhibit caspases 9, 3 and 7. Mesotheliomas have been shown to overexpress the IAPs survivin, XIAP and IAP-1 in vivo using immunohistochemistry [43,65]. IAP-1 has been shown to be associated with shorter survival [44]. RNAi-mediated silencing of IAP-1 is sufficient to reduce mesothelioma cell viability and induce apoptosis by activating the mitochondrial pathway [43]. Conversely, IAP-1, IAP-2 and XIAP are upregulated by tumor necrosis factor alpha, whereas survivin and livin are not [45]. Survivin is overexpressed in mesothelioma and its silencing in vitro is associated with induction of apoptosis suggesting that it might be a potential molecular target [29,144,152].

IAP proteins are inhibited by Smac, which is released from the mitochondria following outer membrane permeabilization by BAX/BAK. Small molecule smac mimetics offer one way of targeting IAPs and are currently in early development, for example, AT406 and TL32711; these compounds also downregulate IAP-1 and IAP-2 [137]. Selective inhibitors of survivin, for example, YM155 are currently in clinical development in other cancers. Other approaches capable of modulating IAP proteins include histone deacetylase inhibition, which is discussed in more detail later in this chapter.

9.3 Tumor Suppressor Loss in Mesothelioma

9.3.1 Loss of nf2 Is Frequent in Mesothelioma

The short arm of chromosome 9 (9p) is a region associated with frequent cytogenetic abnormalities in mesothelioma [19,21,91,100,125]. Loss of the CDKN2b-CDKN2a locus on chromosome 9p21 in humans is a common event in cancer, in general, including mesothelioma. This locus includes the tumor suppressor p16ink4a, which is encoded by CDKN2A and is one of the most frequently silenced tumor suppressors in mesothelioma [53]. This tumor suppressor is an inhibitor of the Rb1 pathway

involved in cell cycle progression, and its loss whether by deletion (75–85%) or methylation is associated with poor prognosis [67,72]. There is frequently co-deletion of p16inka and p15ink4b, occurring in 75% of mesotheliomas [145]. It has been recently shown that p15ink4b, which is encoded by CDKN2b, can substitute for loss of p16ink4a, and that this back-up function could account for the frequently observed loss of the complete CDKN2b-CDKN2a locus [70].

Loss of expression has been shown to be associated with homozygous deletion of exons 1–3 [91,102], and this is more frequently associated with exposure to asbestos even in non-small cell lung cancer, compared with tobacco exposure (which is associated with hypermethylation) [3]. In mesothelioma, hypermethylation occurs in the first exon [142]. Re-expression in mesothelioma cells is sufficient to induce cell cycle arrest, as well as reduced tumor growth and spread in vivo [40,41]. Because hypermethylation silences p16ink4a in approximately 20% of mesotheliomas [142], re-expression can be achieved using demethylating agents such as cytidine analog dihydro-5-azacytidine (DHAC). Analysis of tissue samples from CALGB 8833 and 9031 clinical trials employing DHAC-based therapy identified 4/20 tumors with methylation of p16ink4a. Although there was a trend to improved survival in this clinical trial associated with p16ink4a methylation, this was not statistically significant, probably as a result of the small sample size [69].

Around 40% mesotheliomas harbor somatic mutations in the neurofibromatosis type 2 gene (NF2) located at chromosome 22q12 [116]. Treatment of Nf2 (±) knockout mice with asbestos causes accelerated development of mesothelioma, with biallelic inactivation of the wild-type Nf2 allele, and loss of the CDKN2A locus [1]. Conditional knockout of nf2/p16ink4a in a murine model has been shown to exhibit more invasive, aggressive mesothelioma compared with conditional nf2/p53 knockout, with shorter survival [59]. Together, this implicates an important role in mesothelioma [59]. Mutation of NF2 is frequent in mesothelioma but not observed in non-small cell lung cancer [116]. Somatic mutation of NF2 is conserved across mesothelioma in different species, being frequently detected in murine mesothelioma [73].

9.3.2 NF2 Encodes the Tumor Suppressor Merlin

Merlin, the gene product of NF2 is a FERM domain protein that functions at the plasma membrane where it inhibits mitogenic signaling. It functions as a growth inhibitor, and accumulates in the nucleus where it interacts with and inhibits the E3 ligase CRL4 (DCAF1) [79]. Loss of merlin has a pro-mitogenic effect, and this is lost when DCAF1 is depleted, or if a merlin insensitive mutant is expressed. Mutations of merlin disrupt the direct interaction with CRL4(DCAF1).

When Merlin expression is restored in NF2 deficient mesothelioma cells, there is a marked inhibition of cell motility, spreading and invasiveness. Focal adhesion kinases (FAK) play a critical role in regulating invasive phenotype, and are negatively targeted by merlin. This mechanism of inhibition involves merlin dependent FAK phosphorylation at a critical residue on tyrosine 397, resulting in a block of its interaction with binding partners src and the PI3kinase regulatory subunit p85 [106].

The transcriptional coactivator YAP1 [88] is an oncogene that is commonly amplified at the 11q22 locus in mesotheliomas, and physically interacts with merlin, contributing to the promitogenic effects of NF2 deletion [148]. RNAi-mediated suppression of YAP1 suppresses growth of mesothelioma cells with NF2 homozygous deletion through induction of apoptosis and cell cycle arrest. Conversely, overexpression of YAP1 in immortalized mesotheliomal cells is mitogenic. Merlin inhibits YAP1 through the induction of its phosphorylation and cytoplasmic retention.

9.3.3 PLZF Is a Novel Tumor Suppressor in Mesothelioma

Focal deletion of 11q23 has been identified in mesothelioma, and involves a locus encompassing promyelocytic leukemia zinc finger (PLZF), a transcriptional repressor gene. Loss of PLZF confirmed by analysis of transcript levels, and loss of protein expression has been observed in mesothelioma compared with mesothelial cells. Ectopic expression of PLZF causes reduced clonogenicity and initiation of apoptosis involving caspase activation; together, with the loss of PLZF implicates a potentially important role in regulating mesothelioma cell survival.

9.4 Therapeutic Inhibition of Survival Pathways

9.4.1 PI3K/AKT/mTOR Axis in Mesothelioma

Mesothelioma cells, which have been grown in three dimensions to more closely resemble solid tumors, acquire multidrug resistance, including resistance to TRAIL and chemotherapy [6,62]. The molecular basis underlying acquisition of multidrug resistance has not been fully delineated, but involves activation of the phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway since rapamycin or RNAi silencing of the mTOR target, S6K, can restore TRAIL sensitivity. This effect requires BID, since silencing using RNAi implicates mTOR/S6K as a major contributor of resistance to TRAIL in three-dimensional but not two-dimensional tumors. TRAIL sensitivity is also enhanced by inhibition of the PI3K/AKT pathway following heat stress, supporting a role for this pathway in blocking apoptosis [104]. Mesotheliomas exhibit an elevated level of activity in the PI3K/AKT/mTOR pathway both in mouse and human models, and its inhibition is associated with potentiation of cisplatin-induced apoptosis [2].

AKT is antagonized by the endogenous inhibitor, phosphatase and tensin analog (PTEN), which acts to inhibit phosphorylation. When overexpressed in mesothelioma, PTEN induces loss of viability [87]. Not surprisingly, given the survival function of PTEN in regulating PI3K/AKT/mTOR signaling, the expression is lost in a significant proportion of mesotheliomas [101]. Recently it has been shown that PTEN is required for maintaining the integrity of chromosomes [117]. Loss of PTEN confers a defect in homologous repair which can be exploited by inhibition of poly ADP ribose polymerase (PARP) [85]. Given the recent evidence that PARP inhibitors are very effective in inducing tumor responses under conditions of defective DNA double strand break repair due to BRCA1 mutation [30,37], the possibility exists that a subset of PTEN deficient mesotheliomas may be sensitive to PARP inhibitors.

9.4.2 HGF/cMET Pathway Is Activated in Mesothelioma

C-met receptor tyrosine kinase is overexpressed in mesothelioma by 82% compared with normal tissues, and in 90% of serous effusions [153]. It is associated with high circulating levels of its ligand scatter factor/HGF [57], which in turn is overexpressed in 40–85% of mesotheliomas. HGF stimulates mesothelioma cell motility in vitro via the c-met receptor [49,50,66,128], and has been shown to mediate cell survival by upregulating BCL-XL. The mechanism involves mitogen-activated protein kinase-dependent phosphorylation and activation of the ETS family of transcription factors, which bind to the promoter of BCL-XL [17]. Because phosphorylated c-met and BCL-XL expression are correlated in vivo, it has been proposed that the HGF/met axis mediates survival in part through this interaction at the transcriptional level [17].

The early-response proto-oncogene, fos-related antigen or fra-1 transcriptionally regulates c-met and is upregulated in preclinical models of mesothelioma, as evidenced by expression microarray analysis [109]. Accordingly, HGF-dependent phosphorylation is inhibited by Fra-1 silencing [111]. Fra-1 is a component of the dimeric transcription factor, activator protein-1 or AP-1 and is regulated by phosphatidyl-inositol-3-kinase, extracellular signal-regulated kinases ERK1 and 2, and Src-associated pathways [110]. In addition to c-met being a target of Fra-1, it also directly regulates expression of CD44, the predominant hyaluronic receptor in mesothelioma expression, and thus potentially contributes to control of migration and invasive behavior.

The small molecule c-met inhibitors SU 11274 or PHA-665752, as well as RNAi silencing of c-met, inhibits migration of mesothelioma cells. Susceptibility to c-met inhibition has been reported to depend on the presence of a Met/HGF autocrine loop as evidenced by PHA-665752 [89]. Specific c-met mutations have been identified in two domains; N375S, M431V, and N454I mutations in the semaphorin domain; T1010I and G1085X in the juxtamembrane domain. Interestingly, two mesothelioma cell lines H513 and H2596, which harbor the T1010I mutation, are highly sensitive to SU11274. In addition to c-met mutations, deletion of exon 10 resulting in a splice variant of c-met has been identified in some mesothelioma specimens.

Although activation of the epidermal growth receptor family is observed in mesothelioma, activating mutations of the epidermal growth factor receptor (EGFR) have not been identified in patients with mesothelioma [134]. Targeting EGFR alone in mesothelioma cells has little effect, whereas simultaneous targeting of c-met and EGFR is associated with strong inhibition of proliferation and invasion, suggesting that blocking the coactivation of these two pathways may be more effective than targeting c-met alone [60].

9.4.3 WNT Pathway Activation in Mesothelioma

The Wnt signaling pathways play an important role in homeostasis and development [39]. It suppresses apoptosis through activation of beta-catenin/Tcf-mediated transcription, and is constitutively activated in mesothelioma cells [130]. The canonical Wnt signaling pathway cooperates with loss of NF2 to promote the loss of contact inhibition during proliferation [12]. Gene expression analysis of rat peritoneal mesothelioma induced by o-nitrotoluene or bromochloroacetic acid demonstrates an upregulation of the Wnt/beta-catenin pathway compared with non-transformed mesothelial cells [63]. Using Wnt specific microarray analysis of normal pleura versus mesothelioma, Wnt2 upregulation has been found to be the most common event in mesothelioma [83]. Knockdown of Wnt using RNAi or anti-Wnt2 antibody is sufficient to induce apoptosis, suggesting that Wnt2 could be a potential molecular target [83].

The beta-catenin gene is deleted at 3p21.3 in NCI-H28 cell line [14,118], and this model has been useful in determining the role of beta-catenin-independent Wnt signaling in mesothelioma, via the so-called noncanonical pathway. Wnt inhibitory factor (WIF-1) is a secreted protein that inhibits Wnt signaling and is downregulated in mesotheliomas compared with adjacent pleura [8]. The mechanism of downregulation involves promoter hypermethylation which is seen in malignant, but not adjacent normal pleural tissue. This suggests that epigenetic silencing of WIF-1 could be an important mechanism driving Wnt activation [8]. Similarly, RNAi-mediated knockdown has been shown to suppress cell growth, and colony formation [131]. Secreted Frizzled-related proteins (SFRPs) and the secreted protein dickopf-1 (Dkk-1) are negative regulators of Wnt signaling. SFRPs are silenced by promoter hypermethylation in mesothelioma [74] and re-expression of SFRP4 or Dkk-1 is sufficient to block Wnt signaling in

beta-catenin deficient mesothelioma cells. This implicates a beta-catenin-independent, noncanonical Wnt pathway as a key regulator of cell survival in mesothelioma [51,75,150].

Given the potential importance of Wnt in maintaining mesothelioma cell survival, as well as other cancers (e.g., 80% of colorectal cancers are driven by Wnt mutations [42]), targeting Wnt is a promising strategy. However, no agents have yet entered clinical development. This is because drugging the Wnt pathway has proved difficult. Nevertheless, some small molecules have been identified with the potential to become experimental agents for future clinical studies [20,81]. One promising, but alternative strategy has been to target beta-catenin-mediated transcription. The small molecule XAV939 has been identified by genetic screening. It induces degradation of beta catenin via mechanism involving inhibition of the poly-ADP ribosylating enzymes tankyrase 1 and 2 [54]. This approach might provide a novel strategy for targeting the Wnt pathway in mesothelioma and other cancers.

9.4.4 Estrogen Receptor Beta

Female gender is associated with a favorable prognosis and estrogen receptor beta (ER beta) has been previously shown to be lost in other cancers. This loss is associated with poor prognosis, implicating ER beta as a putative tumor suppressor [7,120]. In mesothelioma, ER beta is downregulated in tumor tissues compared with normal pleura, whereas ER alpha is not expressed [105]. ER beta was recently shown to be an independent prognostic factor for better survival. Activation of ER beta in vitro with 17 beta-estradiol reduces cell proliferation associated with G2/M cell cycle arrest, downregulation of p27, p21, and survivin. These findings suggest that selective estrogen receptor modulators may have a potential role in controlling mesotheliomas.

9.5 Therapeutic Reactivation of Tumor Suppressors

9.5.1 Epigenomic Dysregulation in Mesothelioma

Transformation of normal mesothelium into mesothelioma involves changes to the epigenome. In a study interrogating 1505 CpG loci associated with 803 cancer-associated genes in 158 mesothelioma specimens and 18 normal pleura, the methylation profile was able to effectively discriminate normal pleura from mesothelioma, and was an independent predictor of shorter survival [23]. In an independent study that examined 6157 CpG islands in 20 mesotheliomas in parallel with comparative genomic hybridization and chromatin immunoprecipitation arrays [47], 6.3% of genes were found to be hypermethylated in mesothelioma including MAPK13, KAZALD1, and TMEM30B; 11% of heterozygously deleted genes were affected by DNA methylation and/or H3K27me3. Furthermore, a group of genes silenced by histone H3 lysine 27 methylation (H3K27me3) could be reactivated by histone deacetylation.

Combined epigenetic alterations in mesothelioma are linked with poor prognosis, and these epigenetic alterations may interact cooperatively. In a study, which used nested methylation specific PCR to interrogate the promoter methylation status of nine genes from serum DNA, high incidence of methylation of E-cadherin (71.4%) and FHIT (78%) [36] was measured, whereas intermediate methylation is associated with p16(INK4a) (28.2%), APC1B (32.5%), p14(ARF) (44.2%), and RARbeta (55.8%). Low methylation frequencies were seen for ACP1A (14.3%), RASSF1A (19.5%), and DARK (20%). Interestingly, although no single gene alone predicted survival, combination of RARbeta with either RASSF1A or DARK was associated with significantly shorter survival. This implicates

that silencing of multiple genes can cooperate to influence prognosis in contrast to the effects of these single genes alone.

MicroRNAs are associated with epigenetic regulation. In a study in which 98 mesothelioma specimens were studied using a custom microRNA platform, a training set of 44 tumors and a test set of 98 tumors were analyzed [103]. The microRNA, hsa-miR-29c was shown to be a favorable independent predictor of time to progression and survival after surgical cytoreduction, and was selectively overexpressed in the epithelioid histological subtype. Overexpression of hsa-miR-29c in cell lines was associated with a reduction in clonogenicity associated with reduced proliferation, as well as invasiveness and motility. Epigenetic regulation by hsa-miR-29c was evidenced by its downregulation of DNA methyltransferases and upregulation of demethylating genes, suggesting its role as a prognostic biomarker could relate to its ability to depress transcription of tumor suppressors.

9.5.2 Targeting the Mesothelioma Epigenome via Inhibition of Histone Deacetylases

Histone deacetylases (HDACs) are a class of enzymes that repress genes by inhibiting transcription. As such, they function opposite to histone acetyltransferase which promotes transcription. HDACs remove acetyl groups from ε-*N*-acetyl lysine amino acid on a histone; the effect is to remove the positive charge required for electrostatic interaction with the negatively charged phosphate/DNA backbone, leading to remodeling of chromatin (also termed chromatin expansion), resulting in increased transcription.

HDACs can be selectively inhibited by small molecules [35], and are an active molecular target for clinical development. Mesothelioma cells are sensitive to HDAC inhibition, which can directly modify signaling through the core apoptosis pathway; HDAC inhibition, for example, by sodium butyrate [15,114], causes the downregulation of BCL-XL and induces apoptosis [16]. XIAP is downregulated by HDAC inhibition, and results in increased apoptosis when mesothelioma cells are treated with TRAIL [123]. The HDAC inhibitor Panobinostat (LBH589) is active against mesothelioma cell lines and xenografts [25]. Using a mouse model of B cell lymphoma to explore the proapoptotic pharmacodynamics of vorinostat (suberoylanilide hydroxamic acid or SAHA), the BH3- only proteins BID and BIM were identified as key regulators of intrinsic apoptosis signaling [80]. HDAC inhibition directly downregulates FLIP [18,86,126], with potential to synergize with death receptor agonists [18].

Valproate is an HDAC inhibitor, and has been shown to synergistically interact with cisplatin and pemetrexed in both cell lines, and a xenograft model of mesothelioma [133]. In cells, its cytotoxic activity is associated with activation of both the extrinsic apoptosis pathway, and the intrinsic pathway. Hyperacetylation of histone H3 is induced by valproate consistent with its pharmacodynamics as an HDAC inhibitor. Induction of cell death involves the generation of reactive oxygen species; accordingly, cells can be rescued by the antioxidant *N*-acetylcysteine.

HDAC inhibition may be a promising new development in the treatment of mesothelioma. Although a phase II trial of belinostat (PXD101) which targets class I and II HDACs was shown to be inactive [108], vorinostat exhibited significant activity in a phase I trial, in which monotherapy achieved partial responses [71]. A randomized phase II/III comparing oral vorinostat versus placebo is currently enrolling patients who have relapsed following first line therapy [143]. Given the lack of standard therapy in this clinical setting, this large randomized trial has potential to change practice if it is positive. Recent evidence implicates HR23B as a resistance biomarker of HDAC inhibitors, albeit in cutaneous T cell lymphoma, an indication for which vorinostat has received FDA approval. HR23B shuttles ubiquitinated proteins to the

proteasome. Loss of expression confers resistance to HDAC inhibitors as originally identified by genome-wide RNAi screen. As such, HR23B may represent a potential biomarker for vorinostat in other indications such as treatment of mesothelioma [38,61,113,121,138,151].

9.5.3 Targeting the Ubiquitin Proteasome Pathway

Protein degradation is an essential cellular process which involves tagging with ubiquitin by enzymes called ubiquitin ligases. Proteins are then ferried to the proteasome where degradation to peptides occurs. Small molecule proteasome inhibitors such as bortezomib (velcade) activates BCL-2 family tumor suppressors, leading to induction of apoptosis [33]. These include myc-dependent upregulation of the MCL-1 inhibitor NOXA [34,93,107,139], and other BH3 only proteins such as BIK and BIM [94]. Gene expression studies have implicated dysregulation of the ubiquitin proteasome pathway in mesothelioma [11], and preclinical studies have demonstrated proapoptotic efficacy of proteasome inhibitors in vitro and in vivo [46,113,121,138,151]. This promising activity has led to completion of phase II trials of bortezomib in mesothelioma; EORTC 08052 exploring combination with cisplatin in the first-line setting, and bortezomib monotherapy in the relapsed setting. Mutation and overexpression of proteasome subunit B5 (PSMB5) has been previously identified as a cause of resistance to bortezomib. However, the existence of such mutations in mesothelioma has not yet been established [97].

9.6 Synthetic Lethal Strategies

Mutation of a putative tumor suppressor gene may expose vulnerabilities in a cancer that can be exploited therapeutically. This has been most dramatically demonstrated in the case of somatic BRCA1/BRCA2 mutations, which through inactivation of DNA repair render cancers vulnerable to DNA damage resulting from PARP inhibition [30,85]. Two examples of synthetic lethality associated with dysfunctions in tumor metabolism in mesothelioma will now be considered, where loss of function due to genetic or epigenetic alterations may be exploited, with translation into the clinical setting.

Homozygous codeletion of CDKN2A is frequently associated (90%) with loss of methylthioadenosine phosphorylase (MTAP) [55]. MTAP deficient tumors are responsive to inhibitors of de novo AMP synthesis in the preclinical setting, suggesting a strategy for mediating synthetic lethality. In a multicenter phase II trial to test this concept, patients with MTAP deficient tumors including mesothelioma (as well as non-small cell lung cancer, soft tissue sarcoma, osteosarcoma or pancreatic cancer) were treated with L-alanosine at a dose of 180 mg/m^2 by continuous intravenous infusion daily for 5 out of 21 days. However, no objective responses to therapy were observed leading the investigators to conclude a lack of efficacy [64].

The gene encoding argininosuccinate synthetase (AS), a rate-limiting enzyme involved in arginine metabolism is epigenetically silenced in mesotheliomas, implicating it as a tumor suppressor and highlighting a potential vulnerability which may be exploited therapeutically [26]. AS was shown to be downregulated both in mesothelioma cell lines and a high proportion (63%) of primary mesothelioma specimens [124]. Cell lines lacking AS were unable to synthesize arginine following depletion of arginine from the medium, and underwent apoptosis associated with activation of BAX and mitochondrial depolarization. Silencing of AS was associated with gene methylation.

Induction of apoptosis in AS negative cells following withdrawal of arginine is selective, and not observed in AS positive cell lines, reflecting arginine auxotrophy of AS deficient

cells. Accordingly, lack of AS presents a potential metabolic Achilles' heel in mesothelioma. This phenotype can be targeted pharmacologically, by removing arginine from the circulation using pegylated arginine deiminase, an agent that has received orphan drug status from the FDA for the treatment of hepatocellular carcinoma, and has shown efficacy in melanoma [5,13,28,56]. Because of the high frequency of AS deficiency in mesothelioma, a phase II trial will be evaluating this strategy in patients, tailoring treatment to patients with AS negative mesothelioma [26,124].

9.7 Summary

In recent years, it has become clear that mesothelioma is characterized by frequent activation of survival pathways and inactivation of tumor suppressors. This has opened the door to a growing number of new, rational treatment strategies for targeting vulnerabilities in mesothelioma, that for the first time have real potential for significantly improving treatment response in this chemoresistant cancer, and improving survival outcomes, particularly in the relapsed setting where it is still an unmet clinical need.

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