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Review Article

Tumour suppressor genes in chemotherapeutic drug response

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Synopsis

Since cancer is one of the leading causes of death worldwide, there is an urgent need to find better treatments. Currently, the use of chemotherapeutics remains the predominant option for cancer therapy. However, one of the major obstacles for successful cancer therapy using these chemotherapeutics is that patients often do not respond or eventually develop resistance after initial treatment. Therefore identification of genes involved in chemotherapeutic response is critical for predicting tumour response and treating drug-resistant cancer patients. A group of genes commonly lost or inactivated are tumour suppressor genes, which can promote the initiation and progression of cancer through regulation of various biological processes such as cell proliferation, cell death and cell migration/invasion. Recently, mounting evidence suggests that these tumour suppressor genes also play a very important role in the response of cancers to a variety of chemotherapeutic drugs. In the present review, we will provide a comprehensive overview on how major tumour suppressor genes [Rb (retinoblastoma), p53 family, cyclin-dependent kinase inhibitors, BRCA1 (breast-cancer susceptibility gene 1), PTEN (phosphatase and tensin homologue deleted on chromosome 10), Hippo pathway, etc.] are involved in chemotherapeutic drug response and discuss their applications in predicting the clinical outcome of chemotherapy for cancer patients. We also propose that tumour suppressor genes are critical chemotherapeutic targets for the successful treatment of drug-resistant cancer patients in future applications.

Key words: cancer, chemoresistance, chemosensitivity, clinical prognosis, signal transduction, tumour suppressor gene (TSG)

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INTRODUCTION

Cancer is one of the leading causes of human death worldwide, accounting for 7.6 million deaths each year (World Health Organization, 2008). Various therapies have been developed to treat cancer patients such as radiotherapy, chemotherapy and targeted therapy, biological therapy (immunotherapy) and gene therapy. However, chemotherapy, which randomly kills rapidly growing cancer cells using chemotherapeutics and targeted therapy, which specifically kills cancer cells by targeting oncogenic molecules, are still the most commonly used treatment options for cancer patients. Clinically administered chemotherapeutic drugs are grouped into several families: DNA damaging agents [platinum compounds (cisplatin and carboplatin), anthracyclines (doxorubicin, epirubicin, etc.), alkylating agents (cyclophosphamide, temozolomide, carmustine, etc.) and topoisomerase inhibitors (irinotecan, etoposide, etc.), anti-metabolites [5-FU (5-fluorouracil), methotrexate, capecitabine, etc.], anti-microtubule agents [taxanes (paclitaxel/taxol, docetaxel, etc.) and the *Vinca* alkaloids (vinblastine, vincristine and vindesine)] and oncoprotein targeting agents [humanized monoclonal antibodies such as trastuzumab/herceptin for HER2, cetuximab for EGFR (epidermal growth factor receptor), etc., anti-hormone agents (tamoxifen, flutamide, etc.), and small molecule inhibitors (erlotinib/gefitinib for EGFR, apatinib for VEGFR (vascular endothelial growth factor), etc.)] [1–4]. Although these chemotherapeutics kill cancer cells and

Abbreviations used: ATM, ataxia telangiectasia mutated; JNK, c-Jun N-terminal kinase; 5-FU, 5-fluorouracil; BRCA1$^{-/-}$, breast-cancer susceptibility gene 1 knockout; CDK, cyclin-dependent kinase; CKI, cyclin-dependent kinase inhibitor; CTGF, connective tissue growth factor; EGFR, epidermal growth factor receptor; ESC, embryonic stem cell; HR, homologous recombination; INK4, inhibitor of CDK4; MAD2, myoadenylate deaminase 2; MDR1, multidrug resistance gene 1; MEF, mouse embryonic fibroblast; miR, microRNA; ΔNp63, N-terminal truncated p63; PI3K, phosphoinositide 3-kinase; PIP_2, phosphatidylinositol, 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homologue deleted on chromosome 10; PUMA, p53 up-regulated modulator of apoptosis; Rb, retinoblastoma; siRNA, small interfering RNA; TA, transactivation; TAp63, full-length p63; TAZ, transcriptional co-activator with PDZ-binding motif; TSG, tumour suppressor gene; YAP, Yes kinase-associated protein.

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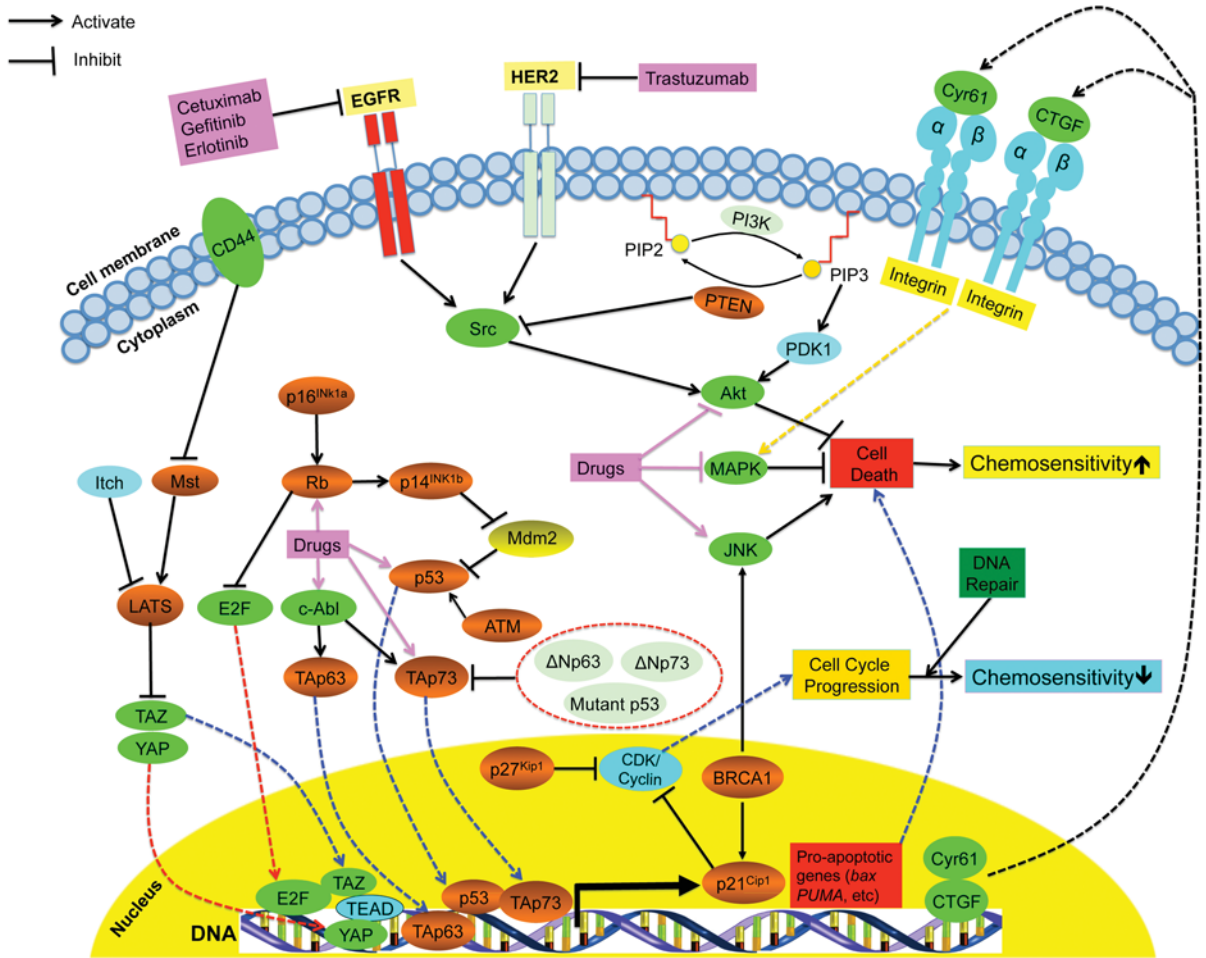

Figure 1 **Signalling pathways mediating tumour suppressor function in chemotherapeutic drug response**
Tumour suppressors are shown in orange and chemotherapeutic drugs are shown in pink. JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase.

can sometimes effectively suppress tumour growth in cancer patients, a significant proportion of tumours either do not respond or later develop resistance to these chemotherapeutics after primary therapy. This leads to tumour recurrence, disease relapse and ultimately patient mortality, which remains a major challenge for successful cancer treatments [2,5–7]. Therefore the identification and characterization of cellular genes responsible for chemotherapeutic drug response is critical for successful prognosis and treatment of cancers. Although many cellular genes, including MDR1 (multidrug resistant gene 1) and c-Myc, have been shown to be involved in the resistance of specific cancer types to some chemotherapeutics [6,8,9], the molecular mechanisms underlying the resistance of distinct types of cancers to different groups of therapeutic drugs remain largely unknown. Most recently, a group of genes called TSGs (tumour suppressor genes) have emerged as important mediators of chemotherapeutic responses. TSGs are frequently dysregulated by mutations or epigenetic modifications in both hereditary cancer syndromes and/or somatically non-hereditary cancers and are also responsible for the initiation and progression of all types of cancers, thereby composing an essential class of signalling molecules within the cell. In this review, we will summarize for the first time the roles of these TSGs in predicting the sensitivity of cancer cells and patients to various chemotherapeutics and their underlying molecular mechanisms. We have also proposed the signalling pathways (Figure 1) illustrating how these TSGs co-ordinately regulate drug sensitivity in cancer cells.

TSGs

Rb (retinoblastoma)

The *Rb* gene was the first TSG originally identified in retinoblastoma [10]. Later studies show that loss of heterozygosity,

down-regulation and mutations of *Rb* have been detected in various human cancers [11–13]. Rb protects against tumorigenesis by regulating cell cycle progression, cellular senescence, differentiation, apoptosis and chromosomal integrity [11,14,15]. Importantly, mounting evidence suggests that Rb status is indicative of predicting chemotherapeutic response. In general, cell culture studies in MEFs (mouse embryonic fibroblasts), MAFs (mouse adult fibroblasts) and human cancer cells (e.g. breast, prostate, lung, etc.) have shown that various chemotherapeutic treatments activate Rb, resulting in cell cycle arrest and activation of DNA repair mechanisms, thereby rendering cells resistant to chemotherapeutics [16–21]. Alternatively, loss of Rb expression in these cell lines using RNAi (RNA interference) bypasses the Rb-induced checkpoint response, sensitizing cells to chemotherapeutic drug-induced apoptosis.

Several mechanisms have been proposed to explain how loss of Rb increases sensitivity to different chemotherapeutics. First, in the absence of Rb, cells continue to replicate unchecked. This continued replication of the damaged genome induced by DNA-damaging chemotherapeutics leads to the accumulation of double strand breaks and enhanced genomic instability [22]. When this DNA damage is irreparable, cells will trigger apoptosis to prevent the propagation of unstable cells. Secondly, it has also been shown that after DNA-damaging drug treatment, the E2F family of transcription factors, normally unrestrained in the absence of Rb, can induce apoptosis by transcriptionally activating pro-apoptotic genes such as the caspases, *APAF1*, and *p73* [23–25]. In addition, DNA damage can also directly activate E2F through ATM (ataxia telangiectasia mutated)/ATR (ataxia telangiectasia mutated- and Rad3-related) and/or Chk2-mediated phosphorylation, thereby stimulating its pro-apoptotic activity [26,27].

Significantly, xenograft mouse models and clinical studies also support these cell line studies showing that loss of Rb increases the sensitivity of tumours to chemotherapeutics regardless of drug class or cancer type. For example, Zagorski et al. [28] demonstrated that tumours in mice xenografted with Rb knockdown lung cancer cell lines (H1299 and H520) regressed significantly when treated with the chemotherapeutics cisplatin, etoposide, or 5-FU compared with their Rb expressing tumours. Similarly, Rb-deficient xenograft mammary tumours responded favourably to cisplatin [19]. Furthermore, clinical studies extend these results demonstrating that Rb-deficient breast cancers treated with chemotherapy are associated with good clinical outcome compared with Rb-proficient breast cancers [20,29–31]. Therefore Rb status can serve as an important marker for predicting chemotherapeutic response.

However, it is worth noting that several studies present conflicting evidence where increased chemoresistance results from Rb deficiency. For example, several studies using sarcoma cell lines show that Rb-deficient cells are resistant to anti-metabolites, topoisomerase inhibitors (etoposide and camptothecin) and DNA damaging agents (doxorubicin and cisplatin) [32–34]. In addition, Rb deficiency in prostate cancer and hepatocellular carcinoma lines are resistant to cisplatin [18,33], whereas Rb-deficient glioblastoma cells are resistant to doxorubicin and etoposide [34].

p53 family (p53, p63 and p73)

p53

p53 is the most frequently mutated TSG in human cancer and is the founding member of the p53 family. In response to various cellular stresses, p53 regulates a variety of cellular functions including cell cycle progression, apoptosis, senescence, cell motility, DNA repair, genetic instability and cell metabolism by transcriptionally activating a variety of cellular genes [35–38]. Significantly, there is evidence from cell, animal and clinical studies that the status of p53 is also associated with cancer cell or patient sensitivity in response to various chemotherapeutics [39–41]. It has been well established that p53 not only induces apoptosis in response to chemotherapeutic drug-induced apoptosis, it can also induce cell cycle arrest, which protects tumour cells from further cytotoxic damage [38]. Despite this apparent discrepancy, in general, studies on various human cancer cell lines demonstrate that cells with mutant p53 are more resistant to drugs compared with those with wild-type p53 when treated with a wide variety of clinically used chemotherapeutic drugs [42–44]. Since p53 induces apoptosis by up-regulating pro-apoptotic genes such as *PUMA* (p53 up-regulated modulator of apoptosis), *Bax*, *Bid* and *Noxa*, it has been demonstrated that loss of p53 many cause drug resistance due to down-regulation of these genes [37–39,45–47].

However, preclinical and clinical studies also suggest that the relative contribution of p53 status to drug response varies depending on cellular context or the class of anticancer drugs used.

In one study, Vasey and Jones [48] showed that inactivation of p53 is associated with reduced sensitivity of ovarian cancer cells to cisplatin but not to the anti-microtubule drug paclitaxel. In addition, p53 disruption rendered colorectal cancer cells resistant to the anti-metabolite 5-FU but sensitized these cells to the DNA damaging drug doxorubicin [49]. Furthermore, the molecular mechanism underlying this distinct role of p53 in the response of different tumour cell types to various drug groups is not fully understood. However, previous studies suggest that diverse functions of p53-induced cell cycle arrest and apoptosis in response to different drugs may contribute to this variability. For example, in response to DNA damaging anthracycline-based therapy, p53 usually activates the apoptotic cascade rather than cell cycle arrest, resulting in tumour regression after drug treatment. However, in response to other drugs such as alkylating agents, p53 may induce cell cycle arrest, which allows the recovery of damaged cells and protect them from drug treatment, resulting in drug resistance and subsequent tumour growth [40]. In addition, p53 can induce cell cycle arrest in ovarian carcinomas, but apoptosis in other cancers after paclitaxel treatments. This may explain why the presence of p53 leads to resistance of ovarian cancer patients to some chemotherapeutic treatments [50]. Therefore the role of p53 in mediating a chemotherapeutic response is complex and depends on both cellular context and class of chemotherapeutics.

p63

p63 is another member of the p53 family. It contains all of the functional domains of p53: an acidic N-terminal TA (transactivation) domain, a highly conserved core DBD (DNA-binding domain) and a C-terminal oligomerization domain. p63 also contains a unique sterile α-motif domain implicated in protein–protein interaction. Besides the TAp63 (full-length p63), p63 can also be expressed as an N-terminal truncated isoform lacking the TA domain [ΔNp63 (N-terminal truncated p63)] transcribed from a second downstream promoter. This ΔNp63 isoform can act as a dominant-negative inhibitor of the full-length TAp63 and p53 [51] and have anti-apoptotic and pro-proliferative functions [52]. Unlike p53, mutations of the p63 gene are rarely detected in human cancer. Instead, down-regulation of p63 has been found to be associated with tumorigenesis and metastasis [53,54]. Most significantly, recent studies show that the level of p63 strongly correlates with the response of tumour cells to chemotherapeutics [55–57]. Chemotherapeutic agents induce TAp63 expression, which subsequently causes apoptosis by directly activating pro-apoptotic genes *CD95*, Bcl-2-family members such as *bax* and *BCL2L11* as well as *Apaf1* [55]. Consequently, inhibition of TAp63 function, which causes reduced apoptosis, leads to drug resistance in various cancers [55,58–60].

Apart from its roles in mediating a chemotherapeutic response, p63 also functions in the drug-induced side effect on fertility. It was recently shown that chemotherapeutic drugs may elicit the death of germ cells, particularly oocytes, through activation of TAp63 rather than p53, which can result in reproductive failure [61]. Most significantly, inhibition of TAp63 activation by blocking its activator c-Abl using an Abl-specific inhibitor imatinib protects mouse oocytes from chemotherapy-induced cell death [62]. Therefore, although inhibition of TAp63 may lead to a limited chemotherapeutic response, its inhibition may also protect from some of the side effect of this treatment, thereby improving the quality of life for patients.

p73

p73 is the third member of the p53 family. Similar to p63, it is also expressed as both a full-length TAp73 (or p73) and a dominant-negative truncated form ΔNp73. Although no mutations in p73 have been detected in human cancers, TAp73 and ΔNp73 are commonly dysregulated [63–67]. Several lines of evidence support a role for p73 in determining chemotherapeutic drug response in cancer treatment. First, TAp73 is induced by diverse groups of chemotherapeutics such as doxorubicin, etoposide, cisplatin and paclitaxel [68–72]. Secondly, p73 knockout ($p73-/-$) MEFs are more resistant to chemotherapeutics compared with their wild-type counterparts [73]. Thirdly, down-regulation of TAp73 caused by overexpression of its dominant-negative mutant ΔNp73 or siRNA (small interfering RNA) leads to enhanced resistance of human tumour cells to chemotherapeutics even when p53 is mutated, whereas overexpression of TAp73 enhances chemosensitivity [66,70,71,74–79]. Finally, these results are supported by *in vivo* clinical data showing that overexpression of the dominant-negative p73 isoform ΔNp73 contributes to drug resistance to platinum-based therapy [80].

Several proteins have been shown to regulate p73 activity in response to chemotherapeutic drug treatments. For example, it has been shown that endogenous p73 is activated in response to a variety of chemotherapeutic drugs in a c-Abl-dependent manner [68,74,81]. Specifically, following drug treatment the tyrosine kinase c-Abl phosphorylates p73 on the tyrosine residue at position 99 (Tyr^{99}) and potentiates p73-mediated transactivation and apoptosis [68,74,81]. c-Abl can also phosphorylate the transcriptional co-activator YAP (Yes kinase-associated protein), which enhances its affinity to p73 and co-activates p73 pro-apoptotic target genes [82]. In addition, chemotherapeutic drug treatment can enhance the interaction between the prolyl isomerase Pin1 and p73 to promote p73 acetylation by acetyltransferase p300, which increases p73 stability and transcriptional activity [83]. Furthermore, it has also been shown both *in vitro* and in clinical cancers that mutant p53, ΔNp63 and ΔNp73 can directly bind and inhibit p73, resulting in reduced transcriptional activation of pro-apoptotic genes *Bax*, *PUMA* and *p53AIP1,* ultimately rendering cancers cells and patients resistant to chemotherapeutic drug treatment [52,70,71,84,85].

CKIs [CDK (cyclin-dependent kinase) inhibitors]

The CKI proteins primarily function as negative regulators of CDKs and cell cycle progression, although each member has also been associated with additional cellular functions, including apoptosis, senescence, transcription, or cell migration [86–88]. CKIs are divided into two families including the INK4 (inhibitor of CDK4) family ($p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$ and $p14^{INK4d}$) and the Cip/Kip family ($p21^{Cip1}$, $p27^{Kip1}$ and $p57^{Kip2}$). INK4 family proteins inhibit CDK4 and CDK6 to prevent G_1–S cell cycle progression, whereas the Cip/Kip family proteins inhibit all CDKs and modulate progression through each stage of the cell cycle [86]. Importantly, several of these CKIs including $p16^{INK4a}$, $p14^{INK4d}$, $p21^{Cip1}$ and $p27^{Kip1}$ are implicated in modulating chemotherapeutic sensitivity.

INK4 family

The INK4a-ARF (ADP-ribosylation factor) locus, encoding both $p16^{INK4a}$ and $p14^{INK4d}$ ($p19^{Arf}$ in mice) through alternative splicing, plays a prominent role in tumour development and is one of the most frequently inactivated TSGs in cancer through a combination of mutations and/or epigenetic silencing mechanisms [87,88]. Together $p16^{INK4a}$ and $p14^{INK4d}$ regulate Rb and p53 respectively [89,90]. Although $p16^{INK4a}$ and $p14^{INK4d}$ function through different signalling pathways, their response to various chemotherapeutics is quite similar.

In response to almost all chemotherapeutics and across all cell lines examined, expression of $p16^{INK4a}$ or $p14^{INK4d}$ increases apoptosis in cell lines with functional p53. For example, ectopic expression of $p16^{INK4a}$ expression in nasopharyngeal cell lines led to an increase in apoptosis in response to the anti-metabolite 5-FU or the DNA cross-linking agent cisplatin [91] and inducible $p16^{INK4a}$ expression in a melanoma cell line increased sensitivity

to the DNA alkylating agent melphalan [92]. Similarly, ectopic expression of $p14^{INK4d}$ in breast carcinoma MCF-7 cells or osteosarcoma U2OS cells enhanced chemosensitivity to cisplatin or doxorubicin [93–95]. On the other hand, down-regulation of either $p16^{INK4a}$ or $p14^{INK4d}$ leads to drug resistance in wild-type p53 models. Specifically, in a mouse lymphoma model, Schmitt et al. [96,97] showed that knockout of either $p16^{INK4a}$ or $p19^{Arf}$ in MEFs leads to resistance to the alkylating agent cyclophosphamide. In addition, loss of $p14^{INK4d}$ in MEFs leads to resistance to doxorubicin [98]. Interestingly, in response to topoisomerase inhibitors, overexpression of either $p16^{INK4a}$ or $p14^{INK4d}$ had no effect on cell death [94,95], suggesting that $p16^{INK4a}$ and $p14^{INK4d}$ are specifically activated in response to certain chemotherapeutics.

Importantly these cell line studies correlate with *in vivo* patient clinical studies. Whereas high $p16^{INK4a}$ predicts a positive response to melphalan treatment in patients with melanoma [92] or 5-FU treatment in colorectal cancer patients [99], ovarian cancer patients with a $p16^{INK4a}$ deletion were less likely to respond positively to cisplatin-based chemotherapy after surgery [100]. In addition, loss of heterozygosity of $p16^{INK4a}$ in childhood B-cell precursor acute lymphoblastic leukaemia predicts for a slower response to induction therapy and poorer prognosis [101]. Thus, expression of $p16^{Ink4a}$ may be a useful marker for predicting chemosensitivity.

Cip/Kip family ($p21^{Cip1}$ and $p27^{Kip1}$)

Due to their role in regulating cell cycle progression in response to DNA damage signals or anti-mitogenic cues, a role for $p21^{Cip1}$ and $p27^{Kip2}$ in mediating a chemotherapeutic response became apparent. In general, increased expression of $p21^{Cip1}$ or $p27^{Kip1}$ leads to chemoresistance, whereas loss of $p21^{Cip1}$ or $p27^{Kip1}$ expression sensitizes cell lines to various chemotherapeutics [102–123]. Studies in patients with lung cancer or acute myelogenous leukaemia also show that high expression of $p21^{Cip1}$ predicts for a less favourable response to their respective chemotherapy regimens [124,125]. However, exceptions to this rule occur. For example, several studies show that in ovarian cancer, head and neck carcinoma, or A549 lung cancer cells, ectopic expression of $p21^{Cip1}$ and/or $p27^{Kip1}$ enhanced cisplatin-induced apoptosis [126–128], although no mechanisms were proposed. In addition, low levels of $p27^{Kip1}$ in ovarian cancer patients correlates with chemoresistance to paclitaxel/cisplatin-based therapy [129], and high levels of $p27^{Kip1}$ were observed in 47% of breast cancer patients who were significantly more susceptible to doxorubicin treatment [130]. Thus, the roles of Cip/Kip in determining chemosensitivity may also depend on cell type and/or cellular localization, as well as the involvement of other signalling mechanisms.

Several mechanisms have been described for how $p21^{Cip1}$ and $p27^{Kip1}$ may inhibit drug-induced cell death. Expectedly, one potential mechanism relies on the primary role of $p21^{Cip1}$ and $p27^{Kip1}$ as CDK inhibitors. For example, in response to the microtubule inhibitor paclitaxel, $p21^{Cip1}$ and $p27^{Kip1}$ induce G_2/M arrest [106,112,115,123]. In particular, paclitaxel treatment of breast cancer cells leads to transcriptional up-regulation of $p21^{Cip1}$, which inhibits Cdc2 (cell division cycle 2)/cyclin B kinase activity, thereby delaying entry into mitosis where the microtubule inhibitor paclitaxel is most active [115,119,123]. Alternatively, when the S phase is perturbed due to DNA damaging agents such as cisplatin or doxorubicin, $p21^{Cip1}$ halts the cell cycle and initiates a repair mechanism, thereby allowing cells to continue proliferating [110,114].

Besides a direct role in mediating cell cycle arrest, both $p21^{Cip1}$ and $p27^{Kip1}$ also participate in the mitochondrial or intrinsic apoptotic pathways. For example, loss of $p21^{Cip1}$ increases $p14^{INK4d}$ and p53 expression. This leads to a reduction in the membrane mitochondrial potential, activation of caspase 9 and an increase in pro-apoptotic Bax with a decrease in the anti-apoptotic Bcl-2 protein [107]. In leukaemia cells, $p21^{Cip1}$ prevents down-regulation of c-IAP1 (cellular inhibitor of apoptosis protein 1), an inhibitor of apoptosis [118], whereas overexpression of $p27^{Kip1}$ inhibits activation of procaspase 3, mitochondrial potential changes and cytochrome *c* release in response to etoposide [104]. Thus, depending on cell type or drug treatment, expression and localization of $p21^{Cip1}$ and $p27^{Kip1}$ can mediate a variety of apoptotic effects leading to enhanced chemosensitivity.

PTEN (phosphatase and tensin homologue deleted from chromosome 10)

PTEN is a dual protein and lipid phosphatase that is commonly mutated in many human malignancies [131–133]. Loss of PTEN results in reduced dephosphorylation of PIP_3 (phosphoinositide 3,4,5-trisphosphate), which allows PI3K (phosphoinositide 3-kinase) to phosphorylate PIP_2 (phosphatidylinositol 4,5-bisphosphate) and enhance levels of PIP_3. PIP_3 induction causes increased cell proliferation and cell migration, cell survival and cell size through activation of downstream proteins such as Akt [134,135]. Recently, several reports have also shown that PTEN plays an important role in the response of human cancer cells to oncoprotein targeting agents. Nagata et al. [136] showed that treatment of HER2-overexpressing breast cancer cells with the HER2-targeting antibody, trastuzumab (herceptin), quickly increased PTEN membrane localization and phosphatase activity by reducing PTEN tyrosine phosphorylation via SRC kinase inhibition. On the other hand, down-regulation of PTEN in breast cancer cells results in trastuzumab-resistance both *in vitro* and *in vivo*. In addition, miR (microRNA)-21 can also cause drug resistance by down-regulation of PTEN [137]. Most importantly, clinical studies demonstrate that PTEN status can be used as a predictive marker for determining breast cancer patient response to trastuzumab. Patients with PTEN-deficient breast cancers had significantly poorer response to trastuzumab and shorter overall survival than those with PTEN expression [136,138]. Later studies demonstrated that PTEN down-regulation can be also used as a biomarker to predict low response to the EGFR inhibitors (cetuximab, gefitinib and erlotinib) for treatment of colorectal and lung cancers [138–140].

Several mechanisms have been proposed to explain the requirement of PTEN in response of cancers to oncoprotein

targeting drug therapy. First, activation of PI3K pathway has been shown to be responsible for loss-of-PTEN-induced drug resistance [141,142]. In support of this notion, PI3K inhibitors can reverse loss-of-PTEN-induced trastuzumab resistance, whereas activation of the components of the PI3K pathway, namely PI3K3A and pAkt, caused trastuzumab resistance [136,138,141,143]. In addition, it has been shown that activation of PI3K induces drug resistance by inducing MRP1 (multidrug resistance protein-1) [144]. Secondly, a recent discovery has uncovered a new mechanism of PTEN in regulating trastuzumab resistance. Zhang et al. showed that the non-receptor tyrosine kinase SRC is a key modulator of trastuzumab response [145]. Activation of SRC by EGFR further activates Akt, which leads to both acquired and *de novo* trastuzumab resistance in breast cancer. Most significantly, they found that PTEN could dephosphorylate and inhibit SRC kinase activity. Therefore loss of PTEN leads to increased phosphorylation/activation of SRC and subsequent trastuzumab resistance, which can be reversed by inhibiting SRC with the SRC-specific inhibitor saracatinib [145].

BRCA1 (breast-cancer susceptibility gene 1)

BRCA1 is frequently mutated in inherited breast cancers [146,147]. Although somatic mutations of BRCA1 are rare in sporadic breast and ovarian cancers, epigenetic down-regulation of BRCA1 is a more frequent event [148,149]. BRCA1 functions as a tumour suppressor by regulating transcription, cell cycle checkpoint and DNA repair [150–152]. Most importantly, BRCA1 plays a significant role in the repair of DNA DSBs (double stranded breaks) through HR (homologous recombination) [153].

The participation of BRCA1 in DNA repair provides a strong rationale for a role of BRCA1 in the response to DNA-damaging drugs. Early studies using BRCA1$^{-/-}$ (BRCA1 knockout) ESCs (embryonic stem cells) showed that BRCA1$^{-/-}$ ESCs were more sensitive to cisplatin, a DNA cross-linking platinum-based drug, compared with WT (wild-type) ESCs [154]. Later studies using BRCA1$^{-/-}$ MEF demonstrated that loss of BRCA1 leads to increased sensitivity to a number of DNA-damaging agents, including the anthracycline doxorubicin, the platinum compound carboplatin, and topoisomerase inhibitors irinotecan and etoposide [155]. Moreover, various other studies further confirmed that while reduction of BRCA1 expression by siRNA results in increased sensitivity, overexpression of BRCA1 causes resistance to platinum compounds and topoisomerase inhibitors [156–158]. Most significantly, clinical studies further establish BRCA1 as a biomarker in predicting the outcome of breast, lung and ovarian cancer treatment with DNA-damage-based therapy [159–163]. Together, data from both *in vitro* and *in vivo* studies suggest that low BRCA1 expression, which causes defects in DNA damage repair, represents tumours with high sensitivity to DNA damaging drugs such as cisplatin and PARP [poly(ADP-ribose) polymerase] inhibitors [156,157,159,164–166]. Interestingly, in contrast with DNA-damaging therapy, preclinical and clinical studies also show that loss of BRCA1 causes resistance of cancer cells to other chemotherapeutics including the anti-microtubule agents paclitaxel and docetaxel as well as targeting agents such as the ER (oestrogen receptor) antagonist tamoxifen [5,160,161,163,167,168,170]. Thus, depending on the type of chemotherapeutics, BRCA1 can have either a positive or negative role in mediating chemosensitivity.

Several studies have elucidated the molecular mechanism underlying the opposing effects of BRCA1 on the response of cancer cells to DNA damaging versus anti-microtubule agents. It has been shown that BRCA1 inhibits DNA damaging drug-induced apoptosis by either transcriptionally activating cell cycle checkpoint genes [p21^{Cip1} and GADD45 (growth-arrest and DNA-damage-inducible protein 45)] or facilitating DNA damage repair processes by interacting with proteins involved in HR (e.g. RAD50/MRE11/NBS1) [171]. Therefore when BRCA1 is inactivated by mutations, deletions or down-regulation, cancer cells will no longer be able to repair the drug-induced DNA damage and will therefore trigger apoptosis, which explains why loss of BRCA1 sensitizes cancer cells to DNA damaging agents. On the other hand, in response to microtubule damage induced by anti-microtubule agents such as paclitaxel or docetaxel, BRCA1 is activated to induce mitotic arrest and apoptosis by transcriptionally activating the spindle assembly checkpoint protein MAD2 (myoadenylate deaminase 2) [172] thereby activating the JNK (c-Jun N-terminal kinase) pathway via direct interaction with the JNK–MEKK3 {MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase] kinase 3} complex [167]. Therefore when BRCA1 is lost cancer cells will no longer activate the mitotic spindle checkpoint protein MAD2 and subsequent activation of the pro-apoptotic JNK pathway, resulting in resistance to anti-microtubule agents.

The Hippo tumour suppressor pathway

The emerging Hippo tumour suppressor pathway was originally identified in *Drosophila* and later in mammals [173–177]. In this pathway, the serine/threonine kinases Mst1/2 (Hippo in *Drosophila*) and LATS1/2 together with an adaptor protein hMOB1 are the core players, which transmit signals from upstream tumour suppressors (Fat4, RASSF1A, Kibra, Merlin, hEx, hWW45, etc.). This inhibits the transcriptional co-activators and oncoproteins YAP (Yes kinase-associated protein) and TAZ (transcriptional co-activator with PDZ-binding motif), resulting in reduced cell proliferation and enhanced cell death through modulation of downstream transcriptional targets [178]. In addition, we and others have also shown that the Hippo tumour suppressor pathway can be negatively regulated by several proteins such as the Itch ubiquitin ligase, HA (hyaluronan) receptor CD44 and p53 regulator ASPP1 [179–182]. Most significantly, recent studies show that the Hippo tumour suppressor pathway plays important roles not only in cancer but also in various biological processes such as organ size control, stem cell renewal and differentiation, tissue regeneration, neuronal dendrite growth and mechanotransduction [174,176,183].

Recently, mounting evidence strongly suggests that the Hippo tumour suppressor pathway may also regulate the response of cancer cells to chemotherapeutics. We have recently shown that

knockdown of both LATS1 and its homologue LATS2 by siRNA causes resistance of HeLa cervical carcinoma cells to paclitaxel [184]. Consistent with our findings, in a screen of shRNAs (short hairpin RNAs) targeting TSGs, LATS1 was identified as one of the genes causing paclitaxel resistance upon knockdown in A549 lung cancer cells [185]. In addition, loss of LATS2 in leukaemic cells renders cells more resistant to the DNA-damaging agents doxorubicin and etoposide through promoting YAP and p73 interaction thereby inhibiting transcription of the pro-apoptotic gene *PUMA* [186]. Moreover, we have recently shown that enhanced levels of TAZ, a substrate and downstream target of LATS1/2 kinases (Figure 1), in breast cancer cells correlates with resistance to paclitaxel [187]. Specifically, overexpression of TAZ in TAZ-low MCF-10A immortalized mammary cells causes resistance to paclitaxel whereas knockdown of TAZ in TAZ-high paclitaxel-resistant MDA-MB231 breast cancer cells sensitizes those cells to paclitaxel [187]. This increased paclitaxel resistance results from TAZ activation of TEAD family of transcription factors, which induces transcription and expression of the extracellular matrix genes *Cyr61* (cysteine-rich 61) and *CTGF* (connective tissue growth factor; Figure 1). Similarly, overexpression of the TAZ paralogue, YAP, has also been shown to cause paclitaxel and cisplatin resistance in mammary and ovarian cells [188,189]. Since LATS1/2 and YAP/TAZ have opposing effects on drug response and LATS1/2 can inhibit YAP/TAZ function through phosphorylation [190–192], we propose that YAP/TAZ-TEAD-Cyr61/CTGF is a novel signalling pathway mediating loss-of-LATS-induced drug resistance in the treatment of human cancers (Figure 1). It has also been shown that loss of other important TSGs in the Hippo pathway such as Mst1, hEx and RASSF1A also leads to drug resistance [193–196], whereas overexpression of CD44 or Itch, the negative regulators of the Hippo pathway, induces drug resistance [180,197]. Interestingly, it has also been shown that CD44 induces resistance of glioblastoma cells to the DNA damaging drugs temozolomide and carmustine by inactivating the Mst/LATS/YAP signalling pathway (Figure 1) [180]. Together, these findings clearly demonstrate that the Hippo pathway plays very important roles in the response of cancer cells to chemotherapeutics.

Other TSGs

Besides the TSGs mentioned above, many other TSGs have also been reported as critical regulators of cancer cells to chemotherapeutics. Loss of the TSGs including *SMAD4* (response to paclitaxel), *LZTS2*, *ST14* and *VHL* increases the cells' sensitivity to different chemotherapeutics [185,199], whereas loss of *ARID1A, Caveolin-1, PDCD4, PCDH10, FBW7, SMAD4 (*response to 5-FU*), FUS1* and *p33ING1b* causes drug resistance [200–207]. In addition, many miRs functioning as TSGs have also been shown to regulate drug sensitivity [208]. For example, down-regulation of Let-7, miR-34, or miR-181 increases chemosensitivity, whereas down-regulation of miR-127 causes chemoresistance [209]. In addition, overexpression of miR-125a-5p increases sensitivity to drugs [210], whereas overexpression of miR-15-5p is associated drug resistance [211,212]. The growing list of tumour suppressor genes and their growing importance for mediating a chemotherapeutic response suggests their importance in regulating tumorigenesis and predicting overall survival.

Interaction of TSGs in chemotherapeutic response

Although different TSGs have distinct biological functions, they can co-operate co-ordinately in response to drugs in common signalling pathways as summarized in Figure 1. For example, p53 may interact with many other TSGs in the chemotherapeutic response [16,19,28,32,34]. First, p53 can modulate the TAp73/TAp63-mediated drug response by activating commonly regulated genes such as pro-apoptotic genes Bax and PUMA or the CKI $p21^{Cip1}$ (Figure 1). In addition, p53 status can be used to predict the clinical outcome of breast cancer patients treated with adjuvant chemotherapy (5-FU, methotrexate and cyclophosphamide) only when Rb is active [213]. However, when Rb is deleted or down-regulated by methylation, p53 status has no predictive value for chemosensitivity in these patients. Furthermore, it has also been shown that the combined status of p53 and ATM are also important in predicting drug sensitivity. While suppression of ATM protects tumours from chemotherapeutic treatment in the presence of p53, suppression of ATM dramatically sensitizes tumours to DNA-damaging chemotherapy when p53 is inactivated (Figure 1) [214]. Moreover, p53 function also dictates how $p16^{INK4a}$ or $p14^{INK4d}$ mediate a chemotherapeutic response. In cell lines without functional p53 caused by mutation or down-regulation, increased expression of either $p16^{INK4a}$ or $p14^{INK4d}$ leads to resistance to different chemotherapeutics including anti-metabolites such as folate antagonists, the anti-microtubule paclitaxel, or the DNA intercalating agent cisplatin [116,215,216]. Since $p14^{INK4d}$ primarily functions as an inhibitor of Mdm2 (murine double minute 2), an inhibitor of p53 (Figure 1) [90], loss of p53 in this scenario provides a clear explanation for the inability of $p14^{INK4d}$ to enhance chemosensitivity [98]. The connection between p53 and $p16^{INK4a}$ is less clear and requires further study. Besides p53 and BRCA1 can also functionally interact with other TSGs such as the other p53 family members or PTEN through the modulation of $p21^{Cip1}$ or JNK in response to chemotherapeutic drugs (Figure 1). All together, the complex interactions between TSGs suggest that modulation of TSGs will likely affect multiple signalling pathways and lead to a complex drug response.

CONCLUDING REMARKS

Through the use of mainly human cancer cell lines and mouse models, numerous studies have provided strong evidence that TSGs can either enhance or reduce the sensitivity of cancer cells to chemotherapeutic drugs depending on the cellular context or class of chemotherapeutics (Figure 1). Although some of the TSGs such as BRCA1 have been used as a biomarker in predicting the outcome of chemotherapeutic drug treatment in cancer patients, most of the findings from cell lines and mouse model

remain to be further verified by large-scale screening of clinical cancer patients treated by chemotherapeutics. Furthermore, the complexity of cellular signalling programmes suggests that single gene biomarkers are insufficient for determining treatment outcome. Instead, understanding the contribution of each key player such Rb, p53, the CKIs, PTEN and the Hippo pathway in context with each other will provide a more reliable predictor for tumour response. We anticipate that in the next decade increasing numbers of TSGs will be established as important prognostic markers in predicting the chemotherapeutic response of cancer patients. This will lead to a promising therapeutic strategy whereby drug-resistant patients can be successfully treated through the modulation of tumour suppressor signalling pathways.

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