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Chemoresistance in solid tumours

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introduction

Drug resistance is a major factor that limits the effectiveness of chemotherapy. Tumours can be intrinsically resistant prior to chemotherapy, or resistance may be acquired during treatment by tumours that are initially sensitive to chemotherapy [1]. Furthermore, in the process of acquiring resistance, the tumour may become cross-resistant to a range of chemotherapies and result in resistance, which ultimately leads to treatment failure in over 90% of patients with metastatic disease [2]. The problem of drug resistance is complex as numerous factors affect drug sensitivity, including: accelerated drug efflux; drug activation and inactivation; alterations in drug target; DNA methylation; processing of drug-induced damage; and evasion of apoptosis (Figure 1). Clearly, strategies to overcome chemoresistance are urgently needed. This article will provide an overview of tumour cell-specific drug resistance mechanisms and highlight examples that have clinical relevance.

drug efflux

Increases in drug efflux are often responsible for enhanced drug resistance and are frequently due to enhanced expression of ATP binding cassette (ABC) transporter proteins, such as P-glycoprotein (Pgp) [3]. This type of resistance can affect a range of chemotherapies with differing mechanisms of action and is termed 'multi-drug resistance'. It has most often been linked to overexpression of Pgp, which has been shown to be overexpressed in many drug-resistant cell lines as well as in a number of solid tumours [4, 5]. The cytotoxic drugs that are most frequently associated with multi-drug resistance are hydrophobic, amphipathic natural products, such as the taxanes, vinca alkaloids, anthracyclines and mitomycin C [5, 6]. Furthermore, the DNA topoisomerase-I inhibitor, irinotecan (CPT-11), and its active metabolite, 7-ethyl-10-hydroxy-campothecin (SN-38), are targets for the ABC transporter proteins [4]. A cell line derived from human erythroleukaemia cells that was selected for resistance to the vinca alkaloid vincristine was found to overexpress Pgp and be cross-resistant to 5-fluorouracil (5-FU), suggesting that 5-FU may also be a target of Pgp [7]. Inhibiting Pgp as a strategy of reversing multi-drug resistance has been extensively studied for more than two decades. First generation Pgp inhibitors showed some initial success in the clinic. Chan and colleagues reported a high cure rate in retinoblastoma patients treated with cyclosporin A in combination with chemotherapy (relapse-free rate of 92% in previously untreated patients) [8]. However, the major limitation for first generation Pgp inhibitors was unacceptable toxicity [5, 9]. Second generation Pgp inhibitors include valspodar (PSC 833), which is a more potent Pgp inhibitor and less toxic than cyclosporin. Valspodar, which is a derivative of cyclosporin D, inhibited Pgp with 10- to 20-fold greater activity than cyclosporin A and has been studied in numerous clinical trials in combination with cytotoxic agents [10–13]. Although, second generation Pgp inhibitors were found to have a better pharmacological profile than first generation compounds, they targeted other ABC transporter proteins and had unpredictable pharmacokinetic interactions. A third generation of Pgp inhibitors have also been developed, which are more specific and do not cause alterations in the levels of co-administered cytotoxic agents. One of the most promising third generation Pgp inhibitors is tariquidar, which binds with high affinity to the Pgp transporter and inhibits its activity [14]. Tariquidar was shown to have no effect on the pharmacokinetics of paclitaxel or doxorubicin when it was given to patients with solid tumours [4]. However, in a phase II trial in chemorefractory breast cancer patients, tariquidar showed limited clinical activity in restoring sensitivity to anthracycline- or taxane-based chemotherapies [15].

drug activation/inactivation

Decreased drug activation can also play an important role in drug resistance. To exert its cytotoxic effects, the antimetabolite 5-FU is converted to its active metabolites by several enzymes, such as thymidine phosphorylase (TP), uridine phosphorylase (UP) and orotate phosphoribosyl transferase (OPRT). The levels of these enzymes have been associated with 5-FU sensitivity [16–18]. Irinotecan must be converted to SN-38 by the enzyme carboxylesterase (CE), the activity of which has been demonstrated to be an important determinant of irinotecan sensitivity [19]. Antifolate drugs such as methotrexate (MTX) and raltitrexed (TDX) are polyglutamated by folypolyglutamate synthase (FPGS), which increases their cellular retention and substrate binding affinity [20]. Decreased polyglutamation has been correlated with antifolate resistance in cell line models [21, 22].

Mechanisms that inactivate drugs can diminish the amount of free drug available to bind its cellular target. For example, more than 80% of 5-FU is catabolised by dihydropyrimidine dehydrogenase (DPD) in the liver before it reaches the tumour [23]. In addition, low levels of DPD mRNA expression in

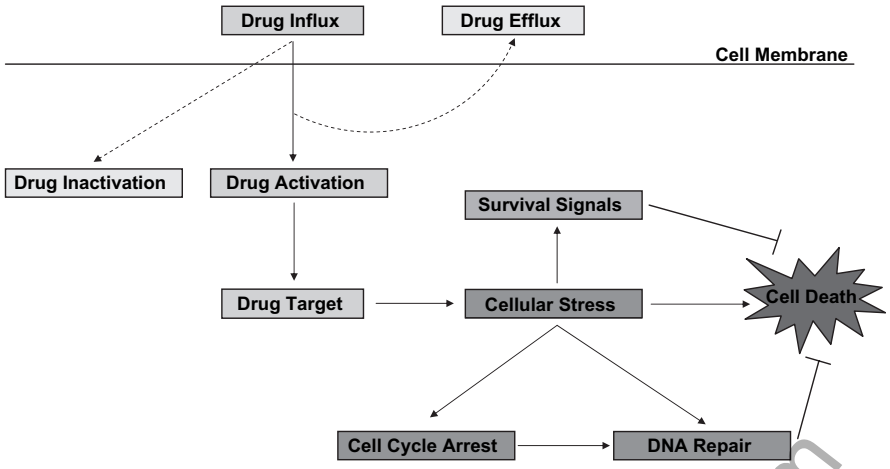

Figure 1. Overview of drug resistance mechanisms in tumour cells.

colorectal tumours have been correlated with sensitivity to 5-FU [24], most likely due to increased 5-FU inactivation in the tumours with high DPD expression. Platinum drugs such as cisplatin and oxaliplatin are able to form conjugates with the thiol glutathione (GSH) resulting in inactivation of these drugs [25]. GSH is a powerful antioxidant that inhibits oxidative stress that can lead to RNA and DNA damage. Platinum drugs become covalently linked to GSH and the resulting complex is a substrate for ABC transporter proteins [26]. Increased levels of GSH have been identified in tumour cells resistant to platinum drugs [27]. GSH conjugation is catalysed by the glutathione-S-transferase (GST) enzyme family, with increased expression of the GST-π subgroup correlating with resistance to cisplatin in ovarian cancer cells [28, 29] and tumours [30]. Inactivation of platinum drugs may also occur via binding to the small, sulphur-rich thiol metallothionein (MT) proteins. Some *in vitro* studies have demonstrated a relationship between MT expression and cisplatin resistance [31, 32]; however, other studies have failed to find any correlation [33, 34]. Therefore, the relationship between MT expression and response to platinum agents remains unclear.

Irinotecan can be inactivated by cytochrome P450 enzymes, and SN-38 is a target for glucuronidation by uridine diphosphogluronysl transferase 1A1 (UGT1A1) [35]. Glucuronidation is a process in which drugs are metabolised to more water soluble compounds and subsequently excreted into the urine or bile. It has been reported that increased glucuronidation may contribute to irinotecan resistance, and polymorphisms in the *UGT1A1* gene have also been associated with irinotecan toxicity [36, 37].

drug targets

Normal, non-mutated drug targets are essential for the maximal effectiveness of chemotherapies. The 5-FU metabolite fluorodeoxyuridine monophosphate (FdUMP) is a potent inhibitor of thymidylate synthase (TS), which is believed to be its primary anticancer mechanism [38]. Preclinical data has demonstrated that TS expression is a key determinant of 5-FU response. *TS* gene amplification, as well as increased TS gene or protein expression, have been shown in cell lines that are resistant to 5-FU [39, 40]. In addition, numerous clinical investigations have measured tumour TS expression by reverse-transcription PCR (RT-PCR) and immunohistochemistry and have demonstrated an enhanced response to 5-FU in patients with low tumoural TS expression [41–43]. More recently, genotyping of the *TS* promoter has divided patients into those who will benefit from a 5-FU-based therapy and those who will not [44]. Polymorphisms in the *TS* promoter can lead to either two (TSER*2) or three (TSER*3) 28 base-pair tandem repeat sequences [45]. Preliminary studies indicate that patients who are homozygous TSER*3/TSER*3 are less likely to respond than patients who are either heterozygous TSER*2/TSER*3 or homozygous TSER*2/TSER*2 [46, 47]. 5-FU treatment has been demonstrated to acutely induce TS expression in both cell lines and tumours [48, 49]. This induction appears to be due to ligand-free TS being able to bind to its own mRNA and inhibit its own translation [50]. However, when bound by FdUMP, TS is unable to suppress its own translation, resulting in increased TS protein expression. If some of the *de novo* synthesised TS protein remains unbound by FdUMP, this may result in resistance to 5-FU.

SN-38, the active metabolite of irinotecan, is a potent inhibitor of topoisomerase-I (topo-I), which is required to relax supercoiled DNA during DNA replication. Topo-I breaks one strand of the DNA helix, allowing the second strand to pass through the cleavage site and finally re-ligates the cleaved DNA [51]. SN-38 stabilises the covalent complex between topo-I and the cleaved DNA, inhibiting re-ligation of the single-strand DNA break. When a replication fork collides with a topo-I/SN-38/DNA complex, double-strand DNA breaks occur [52]. Our laboratory has shown that topo-I mRNA expression was highly down-regulated in an irinotecan-resistant colorectal cancer cell line [53]. Similarly, Jansen et al. found that topo-I activity correlated with sensitivity to irinotecan/SN-38 in a panel of ovarian cancer cell lines [54]. In addition, topo-I mutations have been shown to modulate irinotecan sensitivity both *in vitro* [55] and *in vivo* [56]. Despite these results, the clinical relevance of topo-I expression or mutational status has yet to be elucidated. A second DNA topoisomerase, topoisomerase-II

(topo-II), is also vital during DNA replication. Topo-II is a target for anthracyclines such as doxorubicin and decreased levels of topo-II protein or topo-II mutations have been found in cell lines resistant to topo-II-targeted drugs [57, 58].

DNA methylation

Another factor that may lead to drug resistance is DNA methylation, leading to inactivation of key tumour suppressor genes. DNA methylation occurs by the addition of a methyl group at a 5′ carbon group, usually at cytosine-guanosine dinucleotides (CpGs), resulting in transcription inhibition [59]. Teitz et al. demonstrated that the *caspase-8* promoter was hypermethylated in childhood neuroblastomas and that caspase-8 null neuroblastoma cells were resistant to death receptor- and doxorubicin-induced cell death *in vitro* [60]. In a similar study, Fulda et al. showed that the *caspase-8* gene was hypermethylated in a range of death receptor-resistant solid tumour cell lines, derived from Ewing tumour, neuroblastoma, melanoma and malignant brain tumours [61]. This group further demonstrated that these death receptor resistant cell lines could be sensitised not only to death ligands, but also to various chemotherapies, when pretreated with the demethylation agent 5-Aza-2′-deoxycytidine. Importantly, they showed that the *caspase-8* gene was hypermethylated in a range of primary tumour samples and that this correlated with decreased caspase-8 protein expression. In fact, 5-Aza-2′-deoxycytidine (Vidaza) has recently received FDA approval for the treatment of all subtypes of myelodysplastic syndrome (MDS) [62]. Another gene that is often methylated in brain tumours is the DNA repair enzyme O^6-*methylguanine-DNA methyltransferase* (*MGMT*). A direct relationship between *MGMT* activity and resistance to alkylating agents has been well documented both in cell lines and xenografts derived from gliomas [63]. Clinically, methylation of the *MGMT* promoter was found in 40% of grade III or IV gliomas, which correlated with response to the alkylating agent carmustine. Indeed, 63% of the patients whose tumour's exhibited *MGMT* promoter methylated demonstrated a partial or complete response compared with 4% of patients whose tumour's had unmethylated *MGMT* promoter [64]. Moreover, the lack of *MGMT* promoter methylation correlated with a shorter time-to-progression compared with tumours that had their *MGMT* promoter methylated (8 months compared with 21 months, respectively). In a similar phase II study, Hegi et al. demonstrated that 68% of glioblastomas analysed exhibited methylation of the *MGMT* promoter, and this correlated with a longer overall survival when treated with the alkylating agent temozolomide [65]. These studies demonstrate that hypermethylation of the promoter of the gene encoding *MGMT* is a strong favourable prognostic marker in patients with glioblastomas treated with an alkylating agent. Collectively these results suggest that demethylating agents may improve the effectiveness of chemotherapy in patients whose tumour's primary chemoresistance mechanism results from the silencing of pro-apoptotic genes (such as *caspase-8*) by hypermethylation. However, demethylation may cause expression of previously silenced genes (such as *MGMT*) that may result in increased drug resistance.

DNA damage repair

Many chemotherapeutic drugs cause DNA damage, resulting in cell cycle arrest, DNA damage repair and cell death. Platinum agents result in bulky DNA adducts and are predominantly repaired by the nucleotide excision repair (NER) pathway. Both oxaliplatin- and cisplatin-induced DNA adducts are removed with similar efficiencies by NER [66]. NER is a complex process involving at least 17 different proteins, of which the excision repair cross-complementing 1 (ERCC1) protein is an important rate-limiting factor [67]. In our laboratory, we have demonstrated that ERCC1 mRNA expression was elevated in an oxaliplatin-resistant derived colorectal cancer cell line [53]. Similarly, Hector et al. have shown elevated levels of ERCC1 mRNA in their oxaliplatin-resistant ovarian carcinoma cell line compared to the drug-sensitive parental cell line [68]. Furthermore, ERCC1 downregulation with an antisense expression construct in ovarian cancer cells resulted in increased sensitivity to cisplatin compared with control cells in cell line and xenograft models [69]. In addition, Arnould et al. noted that ERCC1 mRNA expression was predictive of response to oxaliplatin in a panel of colorectal cancer cell lines [70]. Clinically, intratumoural expression of ERCC1 has been correlated with response to platinum-based therapy in ovarian, gastric and non-small-cell lung carcinoma [71–73]. Of note, high mRNA expression of ERCC1 and TS has been shown to be a predictive marker of poor response in patients with advanced colorectal cancer treated with a 5-FU/oxaliplatin combination regimen [74].

Mutations in the DNA mismatch repair (MMR) genes, notably *hMLH1*, *hMSH2* and *hMSH6*, give rise to the microsatellite instability (MSI) phenotype. Mutations in the *hMLH1* and *hMSH2* genes can occur sporadically, or can be inherited, such as in hereditary non-polyposis colon cancer (HNPCC) [75–78]. The MMR system is critical for the maintenance of genomic stability as it scans newly synthesised DNA, excising single-base mismatches and insertion-deletion loops [79]. Resistance to DNA-damaging agents, including platinum drugs, has been associated with MMR deficiency [79, 80]. Cisplatin resistance has been attributed to defects in the MMR system arising from hypermethylation of the *hMLH1* promoter [81]. Interestingly, cell lines which have been shown to be resistant to cisplatin due to MMR defects remain sensitive to oxaliplatin [82, 83]. This observation has been attributed to the structural differences between the cisplatin DNA-adduct and the bulky oxaliplatin DNA-adduct, which are not recognised by the MMR system [84].

p53

Many chemotherapies induce DNA damage, which can cause cell cycle arrest and/or cell death [85]. The tumour suppressor protein, p53, plays a central role in the regulation of cell cycle arrest and cell death [86]. The gene encoding p53, *TP53*, is mutated in approximately 50% of human cancers, making it one of the most frequently mutated genes [87]. In addition, wild-type p53 activity may be compromised by a variety of different mechanisms [88]. DNA damage results in the activation of upstream kinases such as ataxia-telangiectasia mutated (ATM),

ATM- and Rad3-related (ATR) and DNA-dependent protein kinase (DNA-PK) that can directly or indirectly activate p53. Phosphorylation of p53 by these upstream kinases results in enhanced p53 stability and activity by inhibiting its interaction with mouse double minute 2 (Mdm2). Mdm2 is a negative regulator of p53 that targets it for ubiquitin-mediated degradation via the proteasome, thus keeping constitutive levels of p53 low [89]. The role of p53 in determining cell fate following DNA damage is attributed to its role as a transcription factor. p53 transcriptionally upregulates genes that can induce cell cycle arrest, such as $p21^{WAF-1/CIP-1}$ and *GADD45*, thus allowing the cell time to repair the DNA damage [90, 91]. However, p53 can also upregulate genes such as *Fas* (*CD95*) and *Bax* that promote apoptosis, leading to the elimination of cells if the DNA damage is irrepairable [88]. Several experimental reports have indicated that lack of functional p53 contributes to drug resistance, which has been attributed to the inability of the tumour cell to undergo apoptosis. For example, loss of p53 function has been correlated with decreased sensitivity to 5-FU *in vitro* [92, 93]. In colorectal cancer, p53 overexpression, a surrogate marker for mutated p53, correlated with resistance to 5-FU-based therapies [94–96]; however, other studies have failed to find any correlation [97]. This conflicting data may (at least in part) be due to the fact that p53 overexpression does not actually reflect *TP53* mutations in as many as 30%–40% of cases [98]. *In vitro* data correlating p53 status and response to various chemotherapies is unclear. For example, several groups observed decreased sensitivity to cisplatin in p53-mutant cells [99–101], whereas other investigations have shown the opposite [102, 103]. *In vitro* work carried out in our laboratory demonstrated that the isogenic p53 null HCT116 colorectal cancer cell line was less sensitive to 5-FU and oxaliplatin, but not irinotecan compared with the p53 wild-type parental cell line [53]. The topo-II inhibitor doxorubicin was found to be more active in p53 wild-type xenografts compared with p53 mutant or null [104]. In this study 5-FU activity was also enhanced in p53 wild-type xenografts, whereas no difference in cisplatin sensitivity was found. In advanced breast cancer, p53 mutations have been correlated with a lack of response to doxorubicin [105]. Collectively, this data suggests that doxorubicin is more active in p53 wild-type cancers. The antimicrotubule agent paclitaxel has been shown to be equally effective or more effective in p53 null and mutant cell lines than in p53 wild-type cell lines [106–108]. In addition, a study that used paclitaxel in combination with cisplatin was more effective against p53 mutant ovarian tumours than p53 wild-type tumours [109], while another study found that p53 status was not a predictive marker of response to paclitaxel-based regimens in advanced ovarian cancer [110]. Collectively, these clinical studies suggest that p53 status is not a determinant of response to paclitaxel-based chemotherapies.

apoptosis

Defects in cell death signalling are a hallmark of cancer, particularly apoptotic cell death, which is often inhibited in tumour cells due to overexpression of anti-apoptotic proteins (e.g. Bcl-2) or decreased expression of pro-apoptotic proteins (e.g. Fas) [111]. Apoptosis can be initiated via the extrinsic or intrinsic pathways and is mediated by the activation of cysteine proteases (caspases), which are able to amplify the signal, causing cleavage of key proteins, resulting in the morphological changes associated with apoptosis (membrane blebbing, chromatin condensation, DNA degradation).

The extrinsic pathway involves death receptors such as Fas and the TRAIL-receptors (DR4 and DR5), which are members of the tumour necrosis factor (TNF) receptor superfamily. Their predominant function is to induce apoptosis via activation of caspase-8. Typically, activation of death receptors occurs via ligand binding (Fas ligand in the case of Fas and TRAIL in the case of DR4 and DR5) [112]. Activation of death receptors results in the recruitment of an adapter molecule termed Fas-associated death domain (FADD), which can in turn recruit procaspase-8 molecules. This is termed the death inducing signalling complex (DISC), in which caspase-8 is cleaved and activated to initiate apoptosis. p53-responsive elements have been identified in the *Fas*, *DR4* and *DR5* genes [113–115]. Interestingly, p53 has also been implicated in the trafficking of Fas from the Golgi apparatus to the cell surface [116]. We and others have demonstrated that targeting the death receptors with agonistic antibodies or recombinant death ligands can induce apoptosis and enhance chemotherapy-induced apoptosis [117–121]. *In vivo* treatment with Fas monoclonal antibodies resulted in induction of Fas-mediated apoptosis, but also resulted in lethal hepatotoxicity [122]. However, Ichikawa et al. successfully developed a non-hepatotoxic agonistic Fas antibody [123], suggesting that it is possible to develop less toxic Fas antibodies. A more promising clinical approach may be to use TRAIL, as it has been shown to selectively target cancer cells [124]. Several preclinical studies showed that chemotherapy can enhance TRAIL-mediated cell death both *in vitro* and *in vivo* [121, 125–129], and has led the way for a phase I clinical trial. An inhibitor of death receptor-mediated apoptosis termed cellular FADD-like interleukin 1 beta converting enzyme (FLICE)-inhibitory protein (c-FLIP) has been shown to be upregulated in several cancers, including colorectal and gastric cancers [130–132]. We have shown that downregulating c-FLIP expression using small interfering (si) RNAs can enhance chemotherapy-induced cell death in cell-line models [133]. Furthermore, we and others have demonstrated that overexpressing c-FLIP can induce resistance to a range of chemotherapies *in vitro* [133–136]. However, the clinical role of c-FLIP in drug resistance has still to be elucidated.

The intrinsic pathway is regulated by the mitochondria, which are affected early in the apoptotic process and are now known to act as central coordinators of cell death [137]. Several factors can induce mitochondrial-mediated apoptosis, including chemotherapy, ultraviolet rays (UV), DNA damage, reactive oxygen species and growth factor withdrawal. The release of cytochrome c promotes the formation of the apoptosome and activation of caspase-9, the initiator caspase of mitochondrial-mediated apoptosis [138]. Membrane integrity and cytochrome c release are governed by the Bcl-2 family of proteins. Both pro- and anti-apoptotic Bcl-2 family proteins exist, with many of these proteins physically binding to each other, forming a complex network of homo- and heterodimers. It is the relative expression of these proteins to one another that determines cellular fate. Bcl-2 and Bcl-X_L are anchored to the

mitochondrial outer membrane and suppress apoptosis by forming a heterodimer complex with Bax and Bak, preventing the formation of pro-apoptotic homodimers [139]. It is not surprising that regulation of mitochondrial-mediated apoptosis can become disrupted during carcinogenesis. For example, the *Bcl-2* gene has been shown to be overexpressed in many solid tumours cell lines, contributing to resistance to chemotherapy and radiotherapy [140]. Furthermore, it has been demonstrated that downregulation of Bcl-2 and Bcl-X_L using antisense techniques sensitises cells to chemotherapy [141–143], whereas a loss of Bax expression results in increased resistance [144]. Clinically, several studies have shown that high Bcl-2 expression correlates with a poor response to chemotherapy [145–147]. However, overexpression of Bcl-2 in breast carcinomas has been associated with favourable prognostic factors, such as slow proliferation, high steroid receptor levels and absence of p53 overexpression [148]. Loss-of-function mutations have been identified within the *Bax* gene, with more than 50% of microsatellite positive colorectal adenocarcinomas having a frameshift mutation [149]. Some clinical studies have shown a correlation between Bax expression and response to chemotherapy [150, 151]; however, other studies have found no correlation [152, 153]. X-ray crystallography has identified small pockets on Bcl-2 and Bcl-X_L that bind to Bax and Bak. New molecules have been developed to bind to these pockets and inhibit Bcl-2 and Bcl-X_L's antiapoptotic role. An example is the Bcl-2 inhibitor HA14-1, which has been shown to enhance chemotherapy-induced cell death in cell line models [154–156].

Another important mechanism of apoptosis regulation is at the level of caspase activity. Cellular inhibitors of apoptosis proteins (cIAPs) can inhibit apoptosis by binding to active caspases, such as caspase-3, -7 and -9 [157, 158]. Members of this family include cIAP1, cIAP2, XIAP and survivin. IAPs have been shown to be involved in the resistance of certain tumour cells to chemotherapeutic drugs and other apoptosis-inducing agents [159]. Overexpression of survivin has been demonstrated in a wide range of human cancers [160], and survivin expression inhibits chemotherapy-induced cell death *in vitro* [161]. Clinically, low levels of survivin have been correlated with a better response to chemotherapy [162, 163].

survival

Cancer cells have developed various methods by which they can proliferate regardless of their environment. One method includes overexpression of protein tyrosine kinases (PTKs). One of the most well characterised family PTKs is the epidermal growth factor receptor (EGFR) family that includes EGFR [human epidermal growth factor receptor 1 (HER1)], HER2, HER3 and HER4 [164]. Binding of growth factors such as epidermal growth factor (EGF) or transforming growth factor-α (TGF-α), results in the activation of downstream pro-survival and proliferative pathways, such as phosphatidylinositol 3-kinase (PI3-K)/Akt (protein kinase B, PKB), extracellular signal-regulated kinase-1/2 (ERK1/2) and STAT3 and STAT5 (signal transducers and activators of transcription) pathways [164, 165]. Many reports have found EGFR mutations or overexpression in a range of cancers, indicating that it plays an important role in tumourigenesis [165, 166]. Furthermore, overexpresssion of both EGFR and HER2 has been shown to correlate with poor prognosis [167]. In addition, overexpression of EGFR and HER2 have also been shown to increase resistance to chemotherapy *in vitro* [168–170]. HER2 is overexpressed in 15%–25% of breast cancers, resulting in a more aggressive cancer with an increased risk of recurrence. HER2 overexpression has also been correlated with poor clinical outcome, including reduced disease-free and overall survival [171]. Trastuzumab is an antibody that targets and binds with high affinity to the cell surface bound HER2 receptor preventing receptor activation. Clinically, the combination of trastuzumab with chemotherapy in previously untreated patients has been shown to prolong time to progression, increase response rates and significantly improve survival in comparison with chemotherapy alone [172]. Panitumumab and cetuximab are antibodies that selectively bind EGFR, blocking its activation. In a phase II trial, patients with advanced colorectal cancer who had failed 5-FU-based therapies, received panitumumab monotherapy resulting in 10% of patients showing a partial response and 37% of patients maintaining stable disease [173]. In addition, data with cetuximab in combination with irinotecan is encouraging as the combination produced a higher response rate and longer time to progression [174]. The EGFR tyrosine kinase inhibitors gefitinib and erlotinib bind to the cytoplasmic tyrosine kinase domain of EGFR, preventing autophosphorylation and thereby inhibiting EGFR signalling. In a phase II study, 32 patients with advanced colorectal cancer were treated with gefitinib and the FOLFOX (5-FU/oxaliplatin/leucovorin) regimen. Of the patients who had not received any prior treatment, 75% experienced at least a partial response, while in patients who had received prior treatment the response rate was only 23% [175]. Recently, erlotinib in combination with gemcitabine has received Food and drug Administration (FDA) approval in the first-line treatment of patients with metastatic pancreatic cancer. Collectively these results show that targeting the PTK receptors can improve the effectiveness of commonly used chemotherapies. We have found that the phosphorylation status of EGFR determines response to gefitinib alone and in combination with chemotherapy in colorectal cancer cells [176]. However, two large phase II trials (IDEAL-1 and IDEAL-2) showed that gefitinib had only modest antitumour activity as a second- and third-line treatment in patients with advanced non-small-cell lung carcinoma [177, 178]. These discouraging results may be explained as no pre-selection of patients most likely to respond to gefitinib was carried out.

The transcription factor nuclear factor kappa B (NFκB) is normally sequestered in the cytoplasm by inhibitor of kappa B (IκB). Phosphorylation of IκB by IκB kinase (IKK) results in its degradation and allows NFκB to translocate to the nucleus and act as a transcription factor. NFκB is a key regulator of proliferation and inhibitor of apoptosis and exerts its anti-apoptotic activity by upregulating a number of anti-apoptotic proteins, including c-FLIP, cIAPs, Bcl-2 and Bcl-X_L [179]. Chuang et al. demonstrated that a wide range of chemotherapies activated NFκB in a panel of cancer cell lines, indicating that NFκB activation is a key response of a cancer cell to chemotherapy [180]. Moreover, this hypothesis has been corroborated with several *in vitro* studies demonstrating that

inhibition of NFκB activity sensitises cancer cells to chemotherapy-induced cell death [181–183]. Targeting NFκB in conjugation with chemotherapy may prove to be an attractive anti-cancer strategy.

conclusions

Drug resistance is complicated and multifactorial. The development of DNA microarray and proteomic technologies allows us to better understand these genes that may be involved in regulating tumour cell response to chemotherapy. This will facilitate the development of a new generation of molecularly targeted agents designed to increase the effectiveness of chemotherapies. Indeed, basic research has led to the development of many new molecular targeted therapies that have undergone clinical trials with varying degrees of success, examples include gefitinib, erlotinib, cetuximab, panitumumab and trastuzumab, which target HER receptors; genasense (oblimersen) and HA14-1, which target Bcl-2; PRO1762, which targets the TRAIL receptors; and Vidaza, the DNA hypomethylating agent. DNA microarray and proteomic technology will also lead to the identification of panels of biomarkers that will predict response to chemotherapy. Several *in vitro* studies including one of our own have used DNA microarray technology to begin to identify panels of genes, the expression of which may predict response to chemotherapy [184, 185]. In the future, the ability to predict tumour response could enable the selection for the most appropriate chemotherapy and/or molecular targeted regimen for a particular patient.

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