Taxane resistance in breast cancer

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Despite recent progress in the treatment of breast cancer, disease recurrence remains a major issue to curative therapy. One of the major clinical issues is the development of drug resistance. Taxane resistance is a big challenge because taxanes, either alone or in combination, is the first-line regimens for treating breast cancer. A hierarchy of resistant mechanisms are responsible for taxane resistance. At the highest level the mechanisms involve the cancer stem cell (CSC) properties. At the middle level the mechanisms involve the multidrug resistance characters. At the lowest level the mechanisms involve a change of a specific character. Each taxane-resistant case may involve one or more levels of these resistance mechanisms. To overcome taxane resistance, future therapies need to be able to mitigate these three levels of resistance mechanisms.

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Breast Cancer and chemotherapy

Breast cancer is the most common type of cancer in women and there are more than one million reported new cases per year worldwide [1-3]. Among these cases, 20-30% have metastatic or locally advanced disease, and another 30% will develop recurrent or metastatic disease [3]. Metastatic breast cancer (MBC), as a heterogeneous disease, is among the leading causes of cancer mortality [4,5]. Based on the expression of ErbB2 and/or hormone receptors as well as unique gene expression patterns, breast cancer was categorized into five subtypes: luminal like subtype A and subtype B (expression of hormone receptors and luminal cytokeratins 8 and 18), basal-like (expression cytokeratings 5 and 17 and typically no expression of hormone or ErbB2 receptors), ErbB2-positive, and normal-like [5,6].

Chemotherapy is often used to treat breast cancer. For decades, anthracyclines such as doxorubicin have been a standard treatment for MBC. Doxorubicin functions by intercalating with DNA, which leads to chromatin unfolding and aggregation. This chromatin structural disruption is likely to interfere with DNA replication and transcription, which eventually leads to cell apoptosis [7]. It was also suggested that doxorubicin interacts with key cellular enzymes such as topoisomerases II [8-10]. However, cumulative cardiotoxicity limits lifetime exposure to doxorubicin, which prevents doxorubicin from prolonged administrations or re-challenge in later therapy [5,11]. Thus, now the first-line regimens for MBC typically include taxanes such as docetaxel and paclitaxel, either alone or in combination [5,12-15]. Docetaxel and paclitaxel bind to β-tubulin to stabilise microtubules and prevent the
normal formation of mitotic spindles\cite{16}. This causes mitotic arrest\cite{17} and eventually leads to cell death\cite{18}. The other drugs used in treating breast cancer include cyclophosphamide, 5-fluorouracil (5-Fu), capecitabine, and epirubicin \cite{19}. 5-FU works through irreversible inhibition of thymidylate synthase\cite{20}. Capecitabine is converted to 5-FU once taken orally. Cyclophosphamide is a nitrogen mustard alkylating agent and interferes with DNA replication \cite{19}.

**Microtubules and Taxanes**

Microtubules are hollow cylindrical cores composed of α- and β-tubulin heterodimers \cite{21,22}. During the formation of the microtubules, α- and β-tubulin heterodimers are associated together in a head-to-tail fashion to form a microtubule nucleus which elongates linearly into protofilaments. The protofilaments then associate laterally to form microtubules \cite{16,22}. The dynamics of microtubule polymerization and depolymerisation are important in cellular function including cellular division and chromosome segregation during mitosis \cite{22,23,24}. At the beginning of mitosis there are dramatic changes in the microtubule network. After microtubules are disassembled, there is a formation of a new network of spindle microtubules that can turnover up to 100 times more rapidly than interphase microtubules \cite{18,22,23}. Tightly regulated microtubule dynamics is also required for the formation of mitotic asters and kinetochore, which is critical to ensure the correct attachment and segregation of chromosomes during mitosis \cite{16,18}. α- and β-tubulins, microtubule-associated proteins (MAPs), and microtubule-interacting proteins are all involved in the regulation of microtubule dynamics, temporal and spatial organization, and the stability of microtubules \cite{18}.

The taxanes function primarily by interfering with spindle microtubule dynamics by reducing depolymerisation. Stabilization of microtubules by taxane prevents the normal formation of mitotic spindles\cite{16}, which leads to chronic activation of the spindle assembly checkpoint (SAC) and mitotic arrest\cite{17}. Prolonged mitotic arrest eventually leads to cell death\cite{18}. The cellular target for paclitaxel is β-tubulin\cite{21}. Docetaxel shares the same binding site on β-tubulin as paclitaxel, though with greater affinity\cite{25}. The taxane-binding site on microtubule is only present in assembled tubulin\cite{26}.

We have shown previously that treatment with paclitaxel induced a biphasic decrease of viable cells. The first phase of decrease occurs over concentrations ranging from 5 - 50 nM and the second phase of decrease is between the concentrations ranging from 5 - 50 μM. There is a plateau between these two ranges \cite{27,28}. The biphasic response is due to two different mechanisms. In the lower concentration range (5-50 nM), paclitaxel stabilizes the mitotic spindle and arrests the cells at mitosis. This mitotic
arrest results the block of cell proliferation and the induction of apoptosis. In the higher concentration range (5-50 μM), on the other hand, paclitaxel mostly function by increasing the polymerization of microtubule and the formation of microtubule bundles during interphase, which inhibits S phase entry. The inhibition of S phase entry causes the inhibition of cell proliferation and the induction of necrosis [27].

**Taxane Resistance**

Despite recent advances in the treatment of breast cancer, disease recurrence remains a major obstacle to curative therapy [29]. One of the major clinical issues is the development of drug resistance, which accounts for more than 90% of death in patients with MBC [30]. The most established in vitro mechanism for resistance to more than one chemically unrelated class of agents (multidrug resistance) is the overexpression of drug efflux proteins such as ATP-binding cassette (ABC) [1,3,14,22]. Resistance may also arise specific to one group of drug. For example, taxane-resistant cancer cells could have altered expression and function of certain β-tubulin isotypes [1,14,22,28,31-34].

The best known drug efflux proteins are members of the ABC superfamily, including P-glycoprotein [Pgp; also called multidrug resistance protein (MDR) or ABCB1], the multidrug resistance-associated protein 1 [MRP-1, also called ABCC1], and the breast cancer resistance protein [BCRP, also called ABCG2]. ABC transporter substrates include a diverse array of compounds, many of them structurally unrelated. The ABC transporters protect cells by exporting potential toxins, including anticancer agents from normal and cancer cells [14]. In general, ABCB1 transports large hydrophobic compounds, whereas ABCC1 and ABCG2 both transport hydrophobic drugs and large anionic compounds [33]. ABC proteins have been implicated in both taxane and doxorubicin resistance in breast cancers [1,3,14,22]. When 60 cell lines were tested, it was found that the lower the ABCB1 expression level, the greater the sensitivity to paclitaxel in the cell lines [36]. However, in clinic studies the results are controversial. One study shows that increased ABCB1 expression level is correlated with shortened disease-free survival [37]. Some other studies show that no correlation between ABCB expression level and response to taxane treatment in patients with breast cancer [38]. On the other hand, both ABCC1 and ABCG2 mediate resistance to doxorubicin, but not paclitaxel [15,39].

There are seven isotypes of β-tubulin in human [18,40]. While β5-tubulin is only recently been cloned, β4A-tubulin is neural-specific and β6-tubulin is haemopoietic-specific, β1-, β2-, β3-, and β4B-tubulin isotypes have been shown to be expressed in human breast cancer cells [22,28,40]. Structurally, the differences in amino acid sequences among the β-tubulin isotypes are within the 15-20 C-terminal amino acids [41]. The C-terminal region of the β-tubulin isotypes contain the putative binding site for many MAPs [42]. The microtubules of α-tubulin and pure β3 or β4 tubulin require a higher concentration of paclitaxel to induce microtubule stability [43]. The aberrant expression of various tubulin isotypes have been linked to the resistance to taxanes in breast cancers and other cancers [43], It has been reported that both β3- and β4-tubulin are overexpressed in a MCF-7 cell line selected for resistant to paclitaxel under increased paclitaxel concentration [32]. The overexpression of β3-tubulin induces paclitaxel resistance by reducing the ability of paclitaxel to suppress microtubule dynamics [31]. It is also reported that mRNA levels of β2-, β3- and β4-tubulin are significantly up-regulated in paclitaxel- and docetaxel-resistant MCF-7 cells [34]. Abnormal and high levels of expression of β3-tubulin has been associated with more aggressive and taxane-resistant cancers [44].

MAPs including tau, stathmin, MAP2 and MAP4 have also been implicated in taxane-resistance [18,22,45]. They bind to and stabilize microtubules against depolymerisation. High MAP expression is associated with resistance to taxanes [18]. For example, high Tau expression is associated with breast tumors that are resistant paclitaxel [45].

Besides discussed above, many other proteins and mechanisms may also be involved in the acquired drug resistance. It has been shown that proteins related to SACs such as survivin and MDA [22,46,47], cell cycle-related proteins such as p53 [22,48], the membrane receptors such as ErbB2 [22,49], and proteins related to apoptosis such as Bel-2 [22,50,51] are all involved in taxane resistance.

To better understand the mechanisms underlying acquired resistance to taxanes in breast cancer, we recently utilized previously established cell lines in which MCF-7 breast cancer cells were selected for resistance to doxorubicin (MCF-7DOX cells) or docetaxel (MCF-7TXT cells) [28,52]. A cell line selected under identical conditions in the absence of drug (MCF-7CC) was used as a control.

We showed that MCF-7TXT cells that are resistant to docetaxel are cross resistant to other drugs in the same group such as paclitaxel, but are not resistant to doxorubicin, a different type of cancer drug [28]. Our results demonstrate that the acquired taxane-resistance in this instance is specific to the selection agent and it is not a consequence of the establishment of mechanisms of multidrug resistance. It was reported that drug-resistant MCF-7 cells lines also develops cross-resistance to structurally unrelated cancer drugs [34]. However, in this previous report, it is also shown that selected paclitaxel-resistant MCF-7 cell is not cross-resistant to doxorubicin, which is consistent to our data. We further showed that the
selected chemoresistant cell lines do have higher expression level of certain ABC transporter proteins. The expression level of ABCB1 is very high only in MCF-7TXT cells and the expression level of ABCC1 is very high only in MCF-7DOX cells. The expression level of ABCG2 is similar in both the selected chemoresistant and the parental MCF-7 cell lines and likely did not play a role in the taxane-resistant phenotypes of these cell lines [28]. These observations are consistent with a previous report regarding the transcription of these ABC transporter genes in various selected MCF-7 cells [32]. While all of these three ABC proteins have been implicated in multiple drug resistance including taxanes and doxorubicin [1,3,14,22,53], our results suggest that the specific member of ABC transporter proteins that are induced during the selection process may be different depending upon the selection agent. Our results indicate that the resistance to taxanes in MCF-7TXT cells is associated with high expression level of the ABCB1 protein, but not ABCC1 and ABCG2, which is consistent with previous findings in other cell lines [39].

We showed that the acquired resistance of MCF-7TXT cells to taxanes is associated with resistance to taxane-induced apoptosis [28]. While some studies suggest that MCF-7 cells are unable to go apoptosis because of the deletion of caspase-3 gene and thus the lack of caspase-3 protein, other studies indicate that MCF-7 cells are able to go apoptosis through caspase-3 independent apoptotic pathways [54-60]. We further showed that taxane treatment arrested the cells at M phase at the dosage lower than 1 μM, which eventually leads to cell apoptosis.

We studied the effects of taxanes on the formation of microtubules and the mitotic spindles in MCF-7TXT, MCF-7DOX and MCF-7CC cells by indirect immunofluorescence. We showed that the abnormal mitotic spindles induced by taxane treatment are accompanied by the arrest of cells at M phase and the initiation of cell apoptosis (nuclear condensation) [28]. Thus, our data clearly suggests that the acquired resistance to taxanes in MCF-7TXT cells is due to the resistance to taxane-induced mitotic spindle disruption and M phase arrest.

We also examined microtubule dynamics in MCF-7TXT cells and showed that in the absence of docetaxel treatment the microtubule dynamics are robust in both MCF-7TXT and MCF-7CC cells, but the microtubule dynamics are weaker in MCF-7TXT cells than that in MCF-7CC cells. Moreover, microtubule dynamics are greatly more insensitive to docetaxel in MCF-7TXT cells than in MCF-7CC cells [28]. For example, treatment with 0.5 μM docetaxel only slightly reduces the microtubule dynamics in MCF-7TXT cells, but significantly reduced both the shortening and extending rate of microtubules in MCF-7CC cells. Our findings suggest that the resistant MCF-7TXT cells have unique microtubule dynamics that are likely unrelated to the overexpression of ABC transporters. The insensitivity of microtubules to docetaxel treatment in MCF-7TXT cells may be partially the reason that docetaxel is less effective in inducing the M-phase arrest and the apoptosis in MCF-7TXT cells in comparison to MCF-7CC cells. This unique microtubule dynamics may contribute to the resistance to docetaxel.

Finally, we showed that the four β-tubulin isotypes including β1-, β2-, β3- and β4-tubulin are expressed in the three MCF-7 cell lines. While the relative expression levels of the four β-tubulin isotypes are very similar between MCF-7DOX and MCF-7CC cells, the relative expression levels of the β-tubulin isotypes are quite different in MCF-7TXT cells. MCF-7TXT cells have relatively higher β2- and β4-tubulin expression and relatively lower β3-tubulin expression level [28]. These results suggest that the expression level of various β-tubulin isotypes is related to the microtubule dynamics of the MCF-7 cells in response to docetaxel treatment. The expression levels of various tubulin isotypes have been linked to the resistance to taxanes in breast cancers. However, the observation of lower β3-tubulin expression in taxane-resistant MCF-7 cells is contrary to other reports that high β3-tubulin expression is related to taxane-resistance [32]. The overexpression of β3-tubulin induces paclitaxel resistance by reducing the ability of paclitaxel to suppress microtubule dynamics [31]. It is also reported that mRNA levels of β2-, β3- and β4-tubulin are significantly upregulated in paclitaxel- and docetaxel-resistant MCF-7 cells [34]. The significant difference in the expression levels of various β-tubulin isoatypes suggest that the composition of β-tubulin in the formation of microtubules may contribute to the microtubule dynamics and its response to taxane treatment, which could be part of the mechanisms underlying the acquired resistance to taxanes in breast cancer cells.

We also examined the localization of these tubulin isoatypes. We showed that the localization pattern of the various β-tubulin isoatypes in MCF-7TXT cells is different from that of MCF-7CC and MCF-7DOX cells [28]. While we did not know how the different subcellular distribution of these β-tubulin isoatypes affects its response to docetaxel treatment, it is possible that the relative composition of various β-tubulin isoatypes in microtubules and their formation pattern may play a role in determining microtubule dynamics and sensitivity of microtubules to docetaxel treatment.

In conclusion, our results suggest the presence of multiple mechanisms for acquired drug resistance to taxanes. Prolonged exposure to taxanes may result in the selection of the breast cancer cells that overexpress certain drug resistance proteins, such as ABCB1 in MCF-7TXT cells, which will lower the taxane level inside the cells and
thus contribute to the resistance to taxanes. Prolonged exposure to taxanes may also result in the selection of the breast cancer cells that have differential expression of various β-tubulin isotypes, such as higher β-2 and β-4 and lower β-3 tubulin in MCF-7_TXT cells. In addition, the relative composition of various β-tubulin isotypes within the microtubules and the specific distribution of these β-tubulin isotypes along the microtubules may determine the dynamics of the microtubules and its sensitivity to taxane treatment. For example, in MCF-7_TXT cells, the distinct distribution of the β-tubulin isotypes can be related to the weak microtubule dynamics and its insensitivity to taxane treatment.

Cancer stem cells (CSCs)

The CSC concept was based on the evidence that cancer and embryonic tissue share similar capacity to proliferate and differentiate [61]. The CSC hypothesis suggests that the preferential targets of oncogenic transformation are tissue stem or early progenitor cells that have acquired self-renewal potential [62]. It is proposed that the origin of CSC may arise from both intrinsic mutation and extrinsic mutation [63]. CSCs possess the ability to renew and give rise to a progeny that have high proliferative and invasive capacity. This ability is described as “asymmetric division” [64]. The tumors that arise from CSCs consist of CSCs and a mixed population of cells, which makes the tumor highly heterogeneous. CSCs possess some key properties including unlimited proliferative potential and ability to renew; high DNA repair capacity; resistance to cancer therapies; and the ability to drive the expansion of tumor by cells of various differentiation stages [65].

Evidence for CSC has largely relied on primary and early passage xenograft models and immortalized cell lines [62,66,67]. Recent studies suggest that although cell lines may be clonally derived, they contain a cellular hierarchy representing different stages of cellular differentiation. Besides in vitro properties like clonogenic potential, sphere formation, and multilineage differentiation, markers including CD44+/CD24+ and ALDH activity have been used to identify CSCs within breast cancer cell lines [62,66,67]. It has also been suggested that CSC has mesenchymal to epithelial transition (EMT) like gene signature [68]. Moreover, dysregulation of a number of developmental pathways including Wnt, Notch and Hedgehog in the mammary gland generates breast cancers in transgenic mice [69-72]. Furthermore, there is evidence that these pathways are deregulated in many human breast cancers [73-75].

Strategies to overcome drug resistance

The approaches to overcome drug resistance can be more or less classified into two categories, developing new agents and designing new drug administration strategies with existing agents. New agents with high specificity or low susceptibility to resistance mechanisms including trastuzumab, ixabepilone and tariquidar, have developed for effective treatment of breast cancer [76]. On the other hand, application of more than one chemotherapy agents with unrelated modes of action could minimize the impact of drug resistance[5]. Combination chemotherapy kills greater number of tumor cells if the drug dose is not compromised, while sequential chemotherapy allows for greater dose intensity and treatment time for each single agent [77]. However, despite recent progresses in the treatment of breast cancer, drug resistance remains a major obstacle to curative therapy[29]. To overcome drug resistance, several issues in current treatment regimens need to be solved.

First, current treatment regimens are developed with a sole focusing on eliminating the primary cancer without considering how the treatment regimens will affect the development of drug resistance and affect the future treatment of recurrent cancer. As no drug treatment regimens can completely eliminate the cancer cells, it is possible that the few resistant cells surviving the most effective treatment regimens will develop the strongest and broadest resistant against further chemotherapy. Indeed, it is well known clinically that the efficacy in treating recurrent cancer is closely related to the previous exposure to various drugs.

Second, with the recent development of CSC concept, it is recognized that intrinsic properties of CSCs may predispose to tumor relapse [29]. Due to its slow mitotic index, CSCs are less susceptible to chemotherapeutics that target rapidly dividing cells [68]. CSCs also contains considerable variation in genetic materials due to genomic instability, which allow them to survive the various treatment regimens and to develop broad resistance [63]. Thus, it is possible that the current breast cancer treatment regimens are intrinsically deficient as they cannot kill CSCs. Indeed, therapies targeting CSCs may be effective in overcoming drug resistance [29]. Several inhibitors of Notch, Wnt and hedgehog signaling pathways have been tested for their effects on targeting CSCs [69,78-84]. However, our knowledge regarding the origin of CSCs, the differentiation of CSCs and the development of drug resistance in CSCs are still very limited.

Finally, the molecular mechanisms underlying taxane resistance are far from being fully elucidated. For example, while the involvement of β-tubulin in taxane resistance is supported by many researches, the data are very controversial. It is not clear which β-tubulin isotype is critical, how the mutations and expressions of various β-tubulin isotypes affect the taxane-resistance.

To overcome drug resistance, the above issues need to be addressed in the future research. We propose that a
hierarchy of resistant mechanisms exist in taxane-resistant cancer cells including CSC properties, multidrug resistance characteristics, and a specific character against taxanes (Fig. 1). As discussed earlier, the CSC properties include clonogenic potential, sphere formation, multilineage differentiation, CD44+/CD24- and ALDH activity, and EMT like gene signature, as well as dysregulation of a number of developmental pathways including Wnt, Notch and Hedgehog [62,66-72]. The multidrug resistance characteristics include the over expression various ABC proteins, mutation of cell cycle-related proteins such as p53 [22,48], the alterations of the proteins related to apoptosis such as Bcl-2 [22,50,51], and the overexpression of ErbB2 [22,49]. The characters specific to taxanes include mutation and expression of various β-tubulin isotypes [41], MAPs including tau, stathmin, MAP2 and MAP4 [18,22,45], and proteins related to SACs such as surviving and MDA [22,46,47]. The strategies to overcome taxane-resistance have to be able to avoid or mitigate all these three levels of resistance mechanisms. It is important to keep in mind that the cancer treatment regimens including several drugs administrated concurrently or sequentially offers less chance for cancer cells to develop drug resistance; however, once developed the resistance will likely involve the CSC properties and multidrug resistance characteristics. Good treatments regimens need to be effective not only in eliminating the primary cancer cells, but also in reducing the drug resistance, as well as in allowing effective treatment of the recurrent cancer.

Conflicting interests

The authors have declared that no competing interests exist.

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