Class III $\beta$-tubulin expression and in vitro resistance to microtubule targeting agents

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BACKGROUND: Class III $\beta$-tubulin overexpression is a marker of resistance to microtubule disruptors in vitro, in vivo and in the clinic for many cancers, including breast cancer. The aims of this study were to develop a new model of class III $\beta$-tubulin expression, avoiding the toxicity associated with chronic overexpression of class III $\beta$-tubulin, and study the efficacy of a panel of clinical and pre-clinical drugs in this model.

METHODS: MCF-7 (ER+ ve) and MDA-MB-231 (ER− ve) were either transfected with pALTER-TUBB3 or siRNA-tubb3 and 24 h later exposed to test compounds for a further 96 h for proliferation studies. RT–PCR and immunoblotting were used to monitor the changes in class III $\beta$-tubulin mRNA and protein expression.

RESULTS: The model allowed for subtle changes in class III $\beta$-tubulin expression to be achieved, which had no direct effect on the viability of the cells. Class III $\beta$-tubulin overexpression conferred resistance to paclitaxel and vinorelbine, whereas downregulation of class III $\beta$-tubulin rendered cells more sensitive to these two drugs. The efficacy of the colchicine-site binding agents, 2-MeOE2, colchicine, STX140, ENMD1198 and STX243 was unaffected by the changes in class III $\beta$-tubulin expression.

CONCLUSION: These data indicate that the effect of class III $\beta$-tubulin overexpression may depend on where the drug’s binding site is located on the tubulin. Therefore, this study highlights for the first time the potential key role of targeting the colchicine-binding site, to develop new treatment modalities for taxane-refractory breast cancer.

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The high percentage of non-responders and failures following initial responses to taxanes (taxotere and paclitaxel) highlights the critical role played by drug resistance mechanisms in breast cancer progression. Although many mechanisms associated with resistance have been proposed for taxane-refractory cells, only two have been found in the clinic to date: the overexpression of ABC proteins such as the P-glycoprotein (MDR1) and alterations in tubulin isoform expression (Correnti et al, 1995; Linn et al, 1995; Kavallaris et al, 1997; Mozzetti et al, 2002; Paradiso et al, 2005). Mutations within tubulin at the paclitaxel-binding site have been identified (Giannakakou et al, 1997), which were thought to be associated with taxane resistance. However, subsequent studies showed that these early findings resulted from the amplification of tubulin pseudogenes (Noguchi, 2006). Further studies have failed to confirm the presence of tubulin point mutations in patients with lung or ovarian cancers resistant to therapy (Sale et al, 2002; Tsurutani et al, 2002; Lamendola et al, 2003). Microtubule-targeting agents bind to the $\beta$-tubulin subunit of the $\alpha/\beta$-tubulin heterodimers that assemble to form microtubules. In humans, at least seven distinct $\beta$-tubulin isoatypes have been reported. Altered patterns of expression are seen in cancer. In ovarian cancer patients class III $\beta$-tubulin mRNA is significantly upregulated in the taxane-resistant tumours compared with biopsy samples from untreated tumours (Kavallaris et al, 1997). In a separate study, class III $\beta$-tubulin mRNA and protein were shown to be significantly upregulated in a sub-set of paclitaxel resistant ovarian cancer patients (Mozzetti et al, 2005). In breast cancer high expression of class III $\beta$-tubulin is a predictive biomarker of clinical paclitaxel resistance (Tommasi et al, 2007). In gastric cancer patients, whose tumours were positive for class III $\beta$-tubulin expression were significantly less likely to respond to docetaxel-based chemotherapy, 16.7% vs 64.3% response rate, respectively. Therefore, class III $\beta$-tubulin can be regarded as a predictive marker for the clinical response to docetaxel-based chemotherapy in gastric cancer (Urano et al, 2006). The ongoing development of new agents, not sensitive to class III $\beta$-tubulin overexpression, may provide new treatment options for treatment-refractory cancer.

In this study, the effects of seven microtubule targeting agents on the proliferation of MCF-7 and MDA-MB-231 breast carcinoma cells over- or underexpressing class III $\beta$-tubulin were investigated. Five of the agents, 2-methoxyoestradiol-3,17-O, O-bis-sulfamate (STX140), 2-ethyl-oestradiol-3,17-O, O-bis-sulfamate (STX243), 2-methoxyoestradiol (Panzem), colchicine and ENMD1198 are known to target the colchicine-binding site on tubulin, the other two target the taxane-binding site (paclitaxel) and the vinca-binding site (vinorelbine). These seven agents...
represent the currently used drugs in the clinic (paclitaxel and vinorelbine), those drugs that are currently in/have recently completed phase I trials (2-MeOE2 and ENMD1198) and a new generation of orally bioavailable compounds which are in advanced pre-clinical development (STX140 and STX243).

MATERIALS AND METHODS

Drug synthesis

2-Methoxyoestradiol (2-MeOE2, Figure 1 compound I) was synthesised as described previously (Leese et al., 2005a). 2-Methoxy-oestradiol-3,17-O,O-bis-sulfamate (STX140, Figure 1 compound II) and 2-ethylestradiol-3,17-O,O-bis-sulfamate (STX243, Figure 1 compound III) were synthesised by reaction of the appropriate 2-substituted oestradiol in dimethyl acetamide solution with sulphamoyl chloride (Leese et al, 2005b, 2006). 2-Methoxyoestradiol-1,3,5(10),16-tetraene-3-carboxamide (ENMD1198, IRC110160, Figure 1 compound VI) was synthesised as described in US2005/203075 (Foster et al, 2008). Paclitaxel (Figure 1 compound IV, Sigma, Poole, UK), vinorelbine (Figure 1 compound V, Sigma) and colchicine (Figure 1 compound VII, Sigma) were purchased from commercial sources.

Cell culture

MCF-7 (ER+ ve) and MDA-MB-231 (ER–ve) human breast cancer cells were obtained from the American Type Culture Collection (LGC Promochem, Teddington, UK). Cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) foetal calf serum, 1% L-glutamine (200 mM), 1% non-essential amino acids (100×) and 1% bicarbonate (7.5%) from Sigma and maintained in a humidified incubator under 5% CO2 atmosphere at 37°C.
Class III \(\beta\)-tubulin cloning

The protein-expression vector (pALTER-TUBB3) was prepared by inserting the full-length human class III \(\beta\)-tubulin cDNA fragment from pOTB7-TUBB3 (LGc Promochem) into pALTER Max (Promega, Southampton, UK). The construct was checked by digestion and sequencing.

Transfection

MCF-7 and MDA-MB-231 cells were transfected with the wild-type class III \(\beta\)-tubulin (pALTER-TUBB3) or with the class III \(\beta\)-tubulin siGenome Smartpool (siRNA-tubb3, ref. M-020099-03, Dharmacon, Cramlington, UK) using the Amaxa nucleofection technology (Amaxa GmbH, Koeln, Germany). The nucleofection protocol for plasmid transfection was optimised using a GFP-construct with efficacies of 47 and 70% being achieved after 24 h transfection in MCF-7 and MDA-MB-231 cells, respectively. siRNA nucleofection was carried out using a pre-optimised protocol (Mhaidat et al., 2008). Briefly, the cells were trypsinised and washed with PBS. The required number of cells (2 \(\times\) 10^5 cells per nucleofection) were harvested, resuspended in nuclease solution (100 μl per nucleofection) and mixed with 2 μg plasmid or 4 μl of siRNA (20 μM). Cells were then electroporated in the Amaxa nucleofector and transferred to fresh pre-warmed medium. The cells were incubated for 1 h at 37 °C before being seeded in 96-well plates for proliferation assay or in T-25 flasks for protein or RNA extraction. Transfection with the empty pALTER-Max (mock transfection) and a non-targeting siRNA (siGENOME Non-targeting siRNA, ref. D-001210-01, Dharmacon) had no significant effect on the proliferation of either cell line (data not shown).

Proliferation assay

The cells were seeded into 96-well culture plates at 4000 cells per well, incubated at 37 °C for 24 h and exposed to compounds for 96 h at 37 °C. Cell proliferation was measured by adding 10 μl AlamarBlue reagent (BioSource International, Camarillo, CA, USA). The viable cells are counted with a spectrofluorometric AlamarBlue reagent (BioSource International, Camarillo, CA, USA). 1m m l of RNeasy lysis buffer (Qiagen) plus 1% β-mercaptoethanol (Promega) and homogenised with the QiAshredder columns (Qiagen). The RNA was isolated from the homogenate using the RNeasy kit (Qiagen). A 5 μg aliquot of each RNA sample was reverse transcribed using the First-Strand cDNA Synthesis kit (GE Healthcare). Reverse-transcription–PCRs were carried out in a Rotor Gene 2000 Real-time Cycler (Corbett Research, Cambridge, UK) with 2 μl cDNA in a final volume of 25 μl using Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Primers and hydrolysis probes for class III \(\beta\)-tubulin (ref. Hs00964965_m1) and for the internal housekeeping gene, RPLO, (ref. 4310879E) were synthesised by Applied Biosystems. The relative Ct values were calculated using the Rotor Gene 6 software (Corbett Research, Cambridge, UK) and the comparative Ct values were given as:

\[\text{amplification efficiency of target gene} = \frac{(\text{C}_{\text{target}} - \text{C}_{\text{control}})}{(\text{C}_{\text{control}} - \text{C}_{\text{sample}})} \]

\[\text{amplification efficiency of reference gene} = \frac{(\text{C}_{\text{reference}} - \text{C}_{\text{control}})}{(\text{C}_{\text{control}} - \text{C}_{\text{sample}})} \]

Immunohistochemistry

MCF-7 and MDA-MB-231 cells grown on poly-L-lysine-coated coverslips were exposed to compounds for 24 h. The cells were fixed in methanol at −20 °C for 10 min, and then in ice-cold acetone for 30 s. Coverslips were rinsed and then rehydrated with PBS. To visualise the microtubules, cells were incubated for 1 h with an α-tubulin-specific FITC-conjugated antibody (ref. F2168, clone DM1A; Sigma) in PBS containing 1% BSA. Cells were washed twice with PBS and counterstained with Hoechst 33342 at 1 mg ml⁻¹ (Sigma) to stain the nuclei. The coverslips were mounted on slides using DePex mounting medium, observed using a Zeiss inverted microscope (× 200) and analysed with the Axiosview imaging system.

Statistics

In vitro experiments were carried out in triplicate and data presented are representative of three or more independent experiments. All errors shown are the mean ± s.d. The Student’s t-test was used to assess significance.

RESULTS

Class III \(\beta\)-tubulin mRNA expression and protein expression

MCF-7 and MDA-MB-231 cells were transfected with mock vector, non-targeting siRNA (MCF-7-control and MDA-MB-231-control), pALTER-TUBB3 (MCF-7-TUBB3 and MDA-MB-231-TUBB3) or siRNA-tubb3 (MCF-7-siubb3 and MDA-MB-231-siubb3). Gene expression levels of class III \(\beta\)-tubulin were examined by RT–PCR. The mRNA level of class III \(\beta\)-tubulin was 2 ± 0.76-fold increased in MCF-7-TUBB3 cells 24 h after transfection (P < 0.05) and 2.4 ± 0.68-fold increased 5 days after transfection (P < 0.01), compared with MCF-7-mock-transfected cells (Figure 2A). The mRNA level of class III \(\beta\)-tubulin was 4.4 ± 2.6-fold increased in MDA-MB-231-TUBB3 cells 24 h after transfection (P < 0.05)
and was 2.1 ± 0.74-fold higher than the control level 5 days after transfection \((P < 0.05, \text{Figure 2B})\) relative to MDA-MB-231 mock-transfected cells. Transfection with siRNA-tubb3 blocked the class III \(\beta\)-tubulin mRNA expression in both cell lines (Figure 2A and B). Class III \(\beta\)-tubulin mRNA levels were reduced by 50 ± 16% in MCF-7-siTubb3 cells 24 h after transfection \((P < 0.05)\) and by 46 ± 16% 5 days after transfection \((P < 0.05)\). Class III \(\beta\)-tubulin mRNA levels were also reduced by 36 ± 15% in MDA-MB-231-siTubb3 cells 24 h after transfection \((P < 0.01)\), but were not significantly different from control cells 5 days after transfection \((90 ± 47\%)\).

To ensure changes in mRNA level correlated with protein expression, MCF-7 and MDA-MB-231 cells were transfected with either pALTER-TUBB3 or siRNA-tubb3 and the proteins were extracted 24 h and 5 days after transfection. Equal protein amounts were analysed by SDS–PAGE and immunoblotting with the human anti-class III \(\beta\)-tubulin or the human anti-class I–V \(\alpha\)-tubulin. Staining with Ponceau S and immunoblotting with an anti-

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**Figure 2** Class III \(\beta\)-tubulin mRNA and protein expression. (A) MCF-7 and MDA-MB-231 cells were transfected with the mock vector or the non-targeting siRNA (control), pALTER-TUBB3 or siRNA-tubb3 and the expression of class III \(\beta\)-tubulin mRNA was quantified by RT–PCR. Three or four independent experiments \((n = 3–4)\) were carried out in duplicate and the results presented are the average of the Ct values relative to the control Ct values, bars: mean ± s.d., * \(P < 0.05\) and ** \(P < 0.01\). The expression of class III \(\beta\)-tubulin protein, total \(\beta\)-tubulin and total \(\alpha\)-tubulin was analysed in (B) MCF-7 and (C) in MDA-MB-231 24 h and 5 days after transfection by SDS–PAGE and immunoblotting: (1) Control 24 h, (2) pALTER-TUBB3 24 h, (3) siRNA-tubb3 24 h, (4) control 96 h, (5) pALTER-TUBB3 96 h and (6) siRNA-tubb3 96 h.
siRNA-tubb3. The transfection with either pALTER-TUBB3 or with the siRNA did not affect the amount of total β-tubulin expressed in cells (Figure 2B and C). No change was observed for cells transfected with the non-targeting siRNA (data not shown).

**Proliferation assays**

MCF-7 and MDA-MB-231 transfected cells were seeded at 4000 cells per well, incubated for 4 days and the percentage of proliferation was monitored by using the Alamar blue reagent. Class III β-tubulin overexpression or silencing did not significantly affect the viability of the cells (the proliferation of MCF-7-TUBB3, MCF-7-situbb3, MDA-MB-231-TUBB3 and MDA-MB-231-situbb3 cells was 105, 101, 97 and 98% respectively, the proliferation rate of control cells, ns). The cytotoxicity efficacy of STX140, STX243, 2-MeOE2, ENMD1198 and colchicine was not significantly affected by overexpression or silencing of class III β-tubulin (Figures 3 and 4, graphs A, B, D, E and G), and the IC50 values of these compounds were not significantly different from IC50 values calculated in control cells (Table 1a and b). Although MDA-MB-231 cells underexpressing the class III β-tubulin were more sensitive to colchicine than the mock-transfected cells when exposed to high concentrations (0.1–10 μM), the IC50 values in both type of cells were not significantly different (IC50 = 37 nM in MDA-MB-231-mock and IC50 = 29 nM in MDA-MB-231-situbb3, Table 1b).

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**Figure 3**  STX140, STX243, 2-MeOE2, paclitaxel, ENMD1198, vinorelbine and colchicine chemosensitivity in MCF-7 cells. MCF-7 cells were transfected with the mock vector or the non-targeting siRNA (control), (A) pALTER-TUBB3 or siRNA-tubb3 and exposed to STX140, (B) STX243, (C) paclitaxel, (D) 2-MeOE2, (E) ENMD1198, (F) vinorelbine or (G) colchicine for 4 days. The percent proliferation was determined by AlamarBlue assay and results presented are the average of three or four independent experiments (n = 3–4) done in triplicate, *P<0.05 and **P<0.01.
Class III β-tubulin expression altered the sensitivity to paclitaxel in both cell lines (Figures 3C and 4C). MCF-7 and MDA-MB-231 cells overexpressing class III β-tubulin were the less sensitive to paclitaxel, with IC50 values of 0.1 nM and 4 pM, respectively (Table 1a and b). These IC50 values were significantly higher (P < 0.01) than those obtained in untreated MCF-7 and MDA-MB-231 cells (7.2 and 2 pM, respectively). In contrast, MCF-7 and MDA-MB-231 cells underexpressing class III β-tubulin were found to be the most sensitive to paclitaxel. Paclitaxel’s IC50 value was significantly reduced in MCF-7-situbb3 cells (IC50 = 4.8 pM, P < 0.05) compared to the IC50 value in control cells. Despite reducing the paclitaxel concentration to 0.1 pM, no IC50 values could be obtained for the MDA-MB-231 cells underexpressing class III β-tubulin as the inhibition of growth was greater than 50% (47%, Figure 4C). At higher concentrations (1 and 10 pM), the class III β-tubulin silencing rendered MDA-MB-231 cells significantly more sensitive to paclitaxel than the control cells (P < 0.05 and P < 0.01, respectively, Figure 4C).

The modification of the β-tubulin expression profile also altered the efficacy of vinorelbine (Figures 3F and 4F). When MCF-7 and MDA-MB-231 cells were transfected with pALTER–TUBB3, the IC50 values for vinorelbine were higher than the IC50 values in the control cells. Despite reducing the vinorelbine concentration to 0.01 μM, no IC50 values could be obtained for the MDA-MB-231 cells underexpressing class III β-tubulin as the inhibition of growth was greater than 50% (47%, Figure 4F). At higher concentrations (1 and 10 μM), the class III β-tubulin silencing rendered MDA-MB-231 cells significantly more sensitive to vinorelbine than the control cells (P < 0.05 and P < 0.01, respectively, Figure 4F).

Figure 4  STX140, STX243, 2-MeOE2, paclitaxel, ENMD1198, vinorelbine and colchicine chemosensitivity in MDA-MB-231 cells. MDA-MB-231 cells were transfected with the mock vector or the non-targeting siRNA (control), (A) pALTER–TUBB3 or siRNA-tubb3 and treated with STX140, (B) STX243, (C) paclitaxel, (D) 2-MeOE2, (E) ENMD1198, (F) vinorelbine or (G) colchicine for 4 days. The percent proliferation was determined by AlamarBlue assay and results presented are the average of three or four independent experiments (n = 3–4) done in triplicate, *P < 0.05, **P < 0.01 and ***P < 0.001.
transfection renders both cell lines significantly more resistant to paclitaxel, effects on microtubule structure were seen in this study as the concentration of paclitaxel used was still cytotoxic. Further studies are planned to understand the unusual structures observed in the MCF-7 class III β-tubulin overexpressing cells.

**DISCUSSION**

Tubulin-binding agents constitute an important class of compound in chemotherapy, but their clinical success has been compromised by the emergence of drug resistance, which is derived from several mechanisms that can be de novo or acquired. Tubulin-binding agents target the microtubule network and two different resistance mechanisms have been associated to the taxane-resistant phenotype in cancer patients: the overexpression of ATP-binding cassette family of proteins such as P-glycoprotein and alterations in tubulin-isoform expression. Previous studies showed that some compounds such as STX140, are able to inhibit the proliferation of resistant MCF-7 dox cells (P-gly + ve) by arresting them in the G2/M phase (Suzuki et al, 2003) and to block the growth of MCF-7 dox tumours (Newman et al, 2008). This study is the first to report the effects of changes in the tubulin-isoform expression profile on the efficacy of new tubulin-binding agents, including Panzem (2-MeO2), ENMD1198 (currently in phase I clinical trials) and STX140 in MCF-7 and MDA-MB-231 cells. Identifying the role of class III β-tubulin in drug resistance is difficult because of the complexity of tubulin auto-regulation. Expression of β-tubulin isoforms is tightly regulated through co-translational degradation of β-tubulin mRNAs in response to an increase in the level of soluble tubulin (Yen et al, 1988; Theodorakis and Cleveland, 1992). This may explain why overexpression of class III β-tubulin failed to confer resistance to paclitaxel, estramustine and vinblastine, among other drugs tested in prostate cells (Ranganathan et al, 2001). In addition, too high expression of class III β-tubulin in cells can be toxic, further complicating this methodology (Hari et al, 2003). To negate this problem Hari et al (2003) inducibly overexpressed class III β-tubulin in CHO cells, this decreased the paclitaxel suppression of microtubule dynamics and caused a limited resistance to paclitaxel (Kamath et al, 2005). Recently, cervical carcinoma HeLa cells were stably transfected with class III β-tubulin (Joe et al, 2008) and

### Table 1 Average IC50 values of STX140, STX243, paclitaxel, ENMD1198, vinorelbine and colchicine in (a) MCF-7 and (b) MDA-MB-231 cells

| MCF-7 cells Avg IC50 values (µM) | Mock | pALTER-TUBB3 | siRNA-tubb3 |
|---------------------------------|------|--------------|-------------|
| (a)                             |      |              |             |
| STX140                          | 0.98 | 1.06         | 0.92        |
| STX243                          | 0.69 | 0.81         | 0.73        |
| 2-MeOE2                         | 0.99 | 0.97         | 0.98        |
| Paclitaxel                      | 7.2 x 10^-6 | 1 x 10^-4** | 4.8 x 10^-6* |
| Vinorelbine                     | 0.06 | 1**          | 0.01*       |
| ENMD1198                        | 0.57 | 0.61         | 0.45        |
| Colchicine                      | 0.024| 0.029        | 0.029       |

| MDA-MB-231 cells Avg IC50 values (µM) | Mock | pALTER-TUBB3 | siRNA-tubb3 |
|--------------------------------------|------|--------------|-------------|
| (b)                                  |      |              |             |
| STX140                               | 0.05 | 0.08         | 0.07        |
| STX243                               | 0.08 | 0.10         | 0.09        |
| 2-MeOE2                              | 0.99 | 0.98         | 0.99        |
| Paclitaxel                           | 2 x 10^-6 | 4 x 10^-6*** | <1 x 10^-7** |
| Vinorelbine                          | 0.12 | 0.25**       | 0.12        |
| ENMD1198                             | 0.08 | 0.09         | 0.09        |
| Colchicine                           | 0.037| 0.042        | 0.029       |

Cells were transfected with pALTER-TUBB3 or siRNA-tubb3. They were then exposed to STX140, STX243, paclitaxel, STX66, ENMD1198, vinorelbine or colchicine for 4 days and the IC50 values were obtained from a graph of percent proliferation vs inhibitor concentration and calculated using Prism (Graphpad Software). ***P<0.001, **P<0.01 and *P<0.05.
were used to test taccalonolides, which were shown not to be susceptible to class III β-tubulin resistance (Risinger et al., 2008). In this study, class III β-tubulin protein levels was only 1.5-fold increased in cells transfected with pALTER-TUBB3. This may have protected the cells against the toxicity of high expression and enabled them to grow with a similar proliferation rate than control cells. However, the overexpression of class III β-tubulin was significant and this degree of overexpression was sufficient to confer a resistance to paclitaxel. These data are supported by the relatively small increase (five-fold) seen in patients with docetaxel-refractory breast cancer (Hasegawa et al., 2003).

This study demonstrates that a variation in the β-tubulin expression pattern in two breast cancer cell lines does not affect the efficacies of STX140, STX243, 2-MeOE2, ENMD1198 and colchicine, whereas the efficacy of vinorelbine and paclitaxel are altered by both class III β-tubulin overexpression and silencing. These results correlate with previous studies using siRNA approaches which showed that class III β-tubulin induces resistance to paclitaxel, vincristine and DNA-damaging agents in non-small cell lung carcinoma (Kavallaris et al., 1999; Gan et al., 2007).

The key difference between class I and class III β-tubulin is an amino-acid substitution leading to a different three dimensional conformation. Class III β-tubulin, which presents only 92% similarity with the other β-tubulin isoforms, bears an Arg277 instead of the Ser277 present in class I β-tubulin. Ser277 and Arg278 are essential for the stable binding of paclitaxel to its binding site in class I β-tubulin (Lowe et al., 2001). In contrast, the structure of the loop of the class III β-tubulin is altered by the substitution of Ser277 with Arg and thus, prevents a stable paclitaxel binding (Ferlini et al., 2005). It has been shown that Class III β-tubulin also generates more dynamic microtubules and counteracts pro-assembling activity of taxanes at the plus ends of microtubules (Kamath et al., 2005), thereby making microtubules more resistant to the stabilisation of microtubule dynamics operated by taxanes (Hari et al., 2003; Kamath et al., 2005; Gan et al., 2007). In contrast, STX140, STX243, 2-MeOE2, ENMD1198 and colchicine are structurally different to paclitaxel; they bind to the colchicine site and they have a conformation, which may allow the formation of a stable complex with class III β-tubulin. Moreover, these agents depolymerise microtubules, whereas paclitaxel stabilises them. This may be a great advantage when class III β-tubulin is overexpressed and the microtubule dynamic is amplified. However, vinorelbine, which is also a microtubule-destabilising agent but binds to the Vinca alkaloid binding site, has its efficacy reduced by the overexpression of class III β-tubulin in vitro in breast carcinoma cells (as shown in this study) and in small lung cancer cells (Gan et al., 2007) and in vivo (Seve et al., 2005). Therefore, class III β-tubulin resistance might be binding-site related and agents binding to the colchicine-binding site might avoid this resistance. This theory is supported by a recent study, wherein the authors showed that HeLa cells overexpressing class

Figure 5  Fluorescent images of cells overexpressing class III β-tubulin after drug exposure. MCF-7-control, MCF-7-TUBB3, MDA-MB-231-control and MDA-MB-231-TUBB3 cells were treated for 48 h with STX140, paclitaxel or colchicine at concentrations close to IC50 values obtained in control cells and stained with FITC-anti-tubulin (tubulin) and Hoechst 33342 (nucleus). Images were taken using a Zeiss inverted microscope at × 200 magnification.
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