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Enhanced stability of microtubules contributes in the development of colchicine resistance in MCF-7 cells

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ABSTRACT

Understanding the mechanism of resistance to tubulin-targeted anticancer drugs is important for improved chemotherapy. In this work, a colchicine-resistant MCF-7 cell line (MCF-7_{Col30}) was generated by the gradual increment of colchicine treatment and the MCF-7_{Col30} showed ~8-fold resistance towards colchicine. MCF-7_{Col30} cells showed ~2.5-fold resistance against microtubule depolymerizing agents, vinblastine, and nocodazole. In contrast, it displayed more sensitivity towards paclitaxel, a microtubule-polymerizing agent. MCF-7 and MCF-7_{Col30} cells showed similar sensitivity towards cisplatin. Further, the level of P-glycoprotein did not increase in MCF-7_{Col30} cells. MCF-7_{Col30} cells resisted the microtubule depolymerizing effects of colchicine. The time-lapse imaging of individual microtubules in live cells showed that the dynamics of microtubules in MCF-7_{Col30} cells was suppressed as compared to the parent MCF-7 cells. The levels of tubulin acetylation and glutamylation increased in MCF-7_{Col30} cells than the parent MCF-7 cells suggesting that microtubules are stabilized in MCF-7_{Col30} cells. Interestingly, the level of βIII tubulin was increased by 2.3 folds whereas that of βII and βIV tubulin was decreased by 55 and 150%, respectively in MCF-7_{Col30} cells. The results suggested that the changes in the level of β-tubulin isoforms and the post-translational modifications of microtubules altered the stability and dynamics of microtubules and contributed to the development of colchicine-resistance in MCF-7 cells.

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1. Introduction

Microtubule-targeted agents are amongst the chemotherapeutic drugs that have been successfully used for the treatment of a wide variety of tumors [1]. Though microtubule inhibitors, at the beginning of chemotherapeutic treatment, exhibit positive clinical outcome with a significant tumor progression-free survival, eventual development of resistance towards these agents is often observed in several cases [1–3]. The development of resistance is a major obstacle in the long-term use of these drugs in chemotherapy [1–3]. Despite significant advances in understanding the molecular mechanisms of action of microtubule inhibitors, there is only a limited knowledge about the mechanisms underlying the development of resistance against them [4]. The overexpression of *MDR1* gene and the activation of P-glycoprotein are considered as possible reasons for the development of resistance towards several anticancer agents [5–8]; however, they may not contribute significantly to the development of resistance against microtubule-targeting agents [9].

Several experimental systems have shown that mutations in α- or β-tubulin may result in altered ligand binding to microtubules and thus, reduced drug efficacy [10–12]. For instance, mutations (D45Y, C211F, D224N, S234N, and K350N) in β-tubulin and (H283Y, E55K, A383V, and R390C) in α-tubulin increase microtubule stability by promoting interactions between protofilaments, thereby, conferring resistance to colcemid and vinblastine [11]. It is, however, being increasingly realized that tubulin mutations are likely to be rare events and therefore may not be clinically relevant. Finally, given the crucial role of microtubule dynamics in mediating the pharmacological response towards these drugs, one mechanism which has been thought to mediate resistance to tubulin-binding agents is the altered microtubule dynamics due to changes in the expression of tubulin isotypes or of the microtubule-associated proteins [13–16]. In mammalian cells, β-tubulin exists as seven different isoforms. β-tubulin class I is the most predominant one and is constitutively expressed in all

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cell types. Class II, III, IVa, IVb are mainly expressed in neural cells whereas class VI is primarily found in blood cells [17]. In fact, gene expression and immunohistochemical analysis of clinical samples have revealed that abnormal expression of βIII tubulin is closely associated with poor prognosis and development of resistance towards taxanes and vinorelbine in a wide range of tumors [18–20]. Also, the differential expression of β-tubulin isotypes may alter the response of cells towards tubulin-targeted chemotherapeutic agents [2,3]. For instance, the cells resistant to docetaxel have been found to exhibit differential expression of β-tubulin isotypes [2,3,21]. Also, the overexpression of class III β-tubulin in ovarian clear cell adenocarcinoma makes the cells more sensitive to paclitaxel treatment [22].

Colchicine, a potent tubulin inhibitor, has been used as a tool to investigate functional properties of microtubules and is clinically used to treat gout [23]. Several colchicine site binding agents are in different phases of clinical trials for cancer chemotherapy [1]. Colchicine resistance has been attributed to tubulin mutations and overexpression of P-glycoprotein [24,25]. In this work, we established a colchicine-resistant subline MCF-7_{Col30}, by continuously exposing a parental sensitive breast cancer cell line MCF-7 to increasing concentrations of colchicine. The resistant subline MCF-7_{Col30} showed a modest cross-resistance against both vinblastine and nocodazole and more sensitivity towards paclitaxel than MCF-7 cells. Interestingly, cisplatin displayed similar inhibitory effects on both MCF-7 and MCF-7_{Col30} cells. The dynamic instability of microtubules was found to be dampened in the MCF-7_{Col30} cells than the parent MCF-7 cells. Further, β-tubulin isotypes composition was found to be different in MCF-7 and MCF-7_{Col30} cells. The data suggested that an increase in the stability of microtubules and reduced colchicine binding due to the changes in the tubulin isotype composition can produce colchicine resistance in tumor cells.

2. Materials and methods

2.1. Materials

Mouse anti-α-tubulin IgG, Mouse anti-acetyl α-tubulin IgG, Sulforhodamine B, FITC conjugated anti-rabbit IgG were obtained from Sigma, St. Louis, MO, USA. Alexa Fluor 568 goat anti-mouse IgG was purchased from Molecular Probes, Invitrogen, CA, USA. Cell culture flasks were purchased from Nunc, Thermo scientific Roskilde, Denmark. Cell culture plates (96 wells and 24 wells) were obtained from Himedia, Mumbai, India. Other reagents used in this study were of analytical grade and obtained from Himedia, Mumbai, India and Sigma, MO, USA. Antibodies against βI, βII, βIII and βIV tubulin isoforms (Abcam Cambridge, MA, USA) were a kind gift from Dr. Krishanu Ray, Department of Biological Sciences, TIFR, Mumbai, India.

2.2. Chemicals

Colchicine, vinblastine sulphate, paclitaxel, nocodazole and cisplatin were purchased from Sigma, St. Louis, MO, USA.

2.3. Cell culture

MCF-7 (human breast adenocarcinoma) cells were procured from cell repository, NCCS, Pune India. Cells were grown in MEM (Eagle's minimum essential medium, Himedia, Mumbai, India) media supplemented with 10% fetal bovine serum (Biowest, Nuaille, France), 2.2 g/l sodium bicarbonate and 1% antifungal and antibacterial solution (Himedia, Mumbai, India) [26,27]. mtrDNA sequence analysis was done by NCCS to confirm the species and cells were tested free from mycoplasma.

2.4. Development of colchicine-resistant variant MCF-7_{Col30} cells from parent MCF-7 cells

Colchicine-resistant variant of MCF-7 cells was developed by a gradual increment of colchicine from 1 nM to 30 nM in the cultured media. The parent MCF-7 cells were cultured in the presence of 1 nM colchicine. Every week, cells were sub-cultured and increment of 1 nM colchicine was done. Old media was replaced twice a week with fresh media containing the respective concentration of colchicine. The increment of colchicine was done till it reached the concentration of 30 nM colchicine. MCF-7 cells growing in the presence of 30 nM colchicine were named as MCF-7_{Col30} and were used for further characterization. While developing the resistant MCF-7_{Col30} cells, parent MCF-7 cells were grown and sub-cultured parallel to maintain the same passage number. We have used the same passaged parent MCF-7 cells to perform the experiments.

2.5. Cell proliferation assay

To examine the development of fold resistance towards colchicine, nocodazole, vinblastine, paclitaxel and cisplatin, parent MCF-7 cells and its colchicine-resistant variant MCF-7_{Col30} cells were seeded in 96 well cell culture plates at a density of 1 x 10^5cells/ml. Next day, old media were removed and fresh media containing increasing concentrations (5–400 nM) of colchicine were added into the wells. Colchicine treatment was done for 48 h and cells were fixed with TCA after incubation. Effect of colchicine on MCF-7 and MCF-7_{Col30} cells proliferation was determined by standard Sulforhodamine B (SRB) assay [26–28]. To examine the necessity of colchicine for the proliferation of MCF-7_{Col30} cells, the cells were first cultured and sub cultured in the absence of colchicine for 3 weeks. After 3 weeks, cells were again grown in the presence of 30 nM of colchicine and cell proliferation was measured by SRB assay.

2.6. DIC microscopy

To examine the morphology of MCF-7 and MCF-7_{Col30} (0.5 x 10^5 cells/ml) cells in the absence and presence of colchicine, cells were seeded on glass cover slips in 24 well cell culture plate. Next day, cells were incubated with the fresh media without or with 30 nM of colchicine for 24 and 48 h, respectively. After the treatment, the morphology of both cell types was analyzed by differential interference contrast microscopy using Eclipse TE 2000U microscope (Nikon, Tokyo, Japan) at 10x magnification and images were processed by Image-Pro Plus software (Media Cybernetics, Silver Spring, MD).

2.7. Effects of colchicine on cell cycle progression of MCF-7 and MCF-7_{Col30} cells determined by flow cytometry

MCF-7 and MCF-7_{Col30} cells were incubated without or with different concentrations of colchicine for 24 h. After treatment, cells were fixed and stained with Propidium Iodide (PI). DNA contents of PI stained samples were quantified in a flowcytometer (FACS Aria, Beckton Dickenson). The number of cells accumulated in different phases of the cell cycle was analyzed by fitting the data in the Modfit LT program (Verity Softwares, Topsham ME) [27,29].

2.8. Immunofluorescence microscopy

MCF-7 and MCF-7_{Col30} cells (0.5 x 10^5 cells/ml) were seeded on glass coverslips in a 24 well cell culture plate. Cells were incubated with different concentrations of colchicine for 24 h. After

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