Reorganization of actin in K-562 and HL-60 cells treated with taxol

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Influence of taxol, microtubular poison, has been studied on distribution of actin. K-562 and HL-60 cells were treated with taxol in a range of concentrations 0.02–10 μM for 72 hours. The reorganization of F-actin was dependent on its dose. Phalloidin conjugated to TRITC was used to evaluate actin distribution by classical fluorescence and confocal microscopy. Actin was visualized at ultrastructural level by using a postembedding streptavidin-gold method. The treatment of K-562 and HL-60 cells with 2–10 μM of taxol resulted in an increase of F-actin in the cytoplasm, with intense labeling as a ring close to surface of the cell. In HL-60 cells a concentration of F-actin at the site of apoptotic bodies was often observed. Immunogold labeling of actin was localized in the nuclei and cytoplasm in control cells and cells treated with all doses of taxol. At higher doses, compaction of chromatin in the nucleus with strong actin labeling was observed. These observations at the ultrastructural level suggest actin involvement in chromatin reorganization during the process of apoptosis. The present study demonstrated a dose dependent reorganization of actin after treatment with taxol.

Key words: taxol, apoptosis, HL-60, K-562, actin, immunogold method

Actin is one of the major constituents of the cytoskeleton in eukaryotic cells and is functionally involved in cell locomotion, membrane ruffling and formation of lamellipodia [1]. A large number of cellular processes, including cytokinesis, endocytosis and chemotaxis are mediated by polymerization of actin filaments [2]. There are also reports on actin involvement in apoptosis [3–10]. Although actin has been studied extensively for decades, every well-defined role found so far has been localized to the cytoplasm [11]. There are also numerous reports on the presence of actin in the nucleus but the role of actin in the nucleus is still unknown [12–18]. Some observations suggest that actin and actin-related proteins play roles in chromatin remodeling and other processes in the nucleus [11, 19–21]. ZHAO and colleagues [21] demonstrated by purification of the mammalian SWI/SNF-like BAF complex that β-actin was a component of the complex *in vivo*. The observation showed that actin might have a direct function in reorganization of chromatin as a part of the SWI/SNF-like BAF complex. GONSIOR et al [22] suggest that there is a structural difference between cytoplasmic and nuclear actin. Here, we used taxol (paclitaxel), which enhances tubulin polymerization [23–25] and prevents microtubule depolymerization and also cell death by apoptosis or necrosis, dependent on drug concentration [26–28]. It acts as a mitotic inhibitor blocking cells in the G2/M phase of the cell cycle [26,29] and is an active cancer chemotherapeutic agent used in a variety of human tumors [30–33]. In our previous studies with other cytostatic drugs we showed alterations in expression of actin especially in HL-60 cells. These changes were dose-dependent and associated with characteristic features apoptosis. At the ultrastructural level, there was increase of positivity for actin in relation with blebbing, margination of nuclear chromatin and bodies containing recognizable nuclear fragments [6, 7]. Actin and microtubules are tightly coordinated by number of proteins, which are implicated in the interaction between these systems [34]. Although, actin-targeted inhibitors are currently not used in the clinic, this system is worthy of study and could be considered in relation to anticancer drugs.

Material and methods

We studied the effect of various concentrations of taxol on reorganization of actin in K-562 (ATCC, CCL 243) and

HL-60 (ATCC, CCL 240) cell lines. For experimental studies, cells were diluted to 5x105 cells/ml and were grown in RPMI 1640 medium supplemented with 10% foetal calf serum and 20 μl gentamycin in a fully humidified atmosphere of 5% CO_2 at 37 °C. The cells were incubated with 0.02, 0.2, 2, 5, 10 μM of taxol (Sigma) for 72 hours. Control cells incubated in absence of taxol were treated identically. Cell viability was assessed by the trypan blue dye exclusion method. F-actin filaments in cells were labeled with phalloidin conjugated to rhodamine (TRITC; Sigma) as previously described [35]. Then cells were collected directly onto microscopical slides using a cytocentrifuge and were fixed with 4% paraformaldehyde for fluorescence microscopy. Then, they were incubated in 2 μM rhodamine-phalloidin calcium and magnesium free in phosphate-buffered saline (PBS-A) containing 20% methanol for 20 min at room temperature in the dark, rinsed twice in PBS-A and mounted in gelvatol (Monsanto, USA). The organization of F-actin filaments was examined with an Eclipse E600 microscope equipped with Y-FL epifluorescence equipment (Nikon, Japan) and the nucleus imaged by using confocal microscope (inverted microscope, Nikon, Eclipse TE 300). Image analysis was performed using Laboratory Universal Computer Image Analysis for fluorescence microscopy and Lucia DI and EZ 2000 ver. 2.5 154 software for confocal microscopy. Mayer's hematoxylin was used to define apoptotic cells. The criteria for apoptosis were: nuclear fragmentation, cell shrinkage with the formation of membrane blebs and buds. To demonstrate actin at the ultrastructural level, a postembedding streptavidin-gold method was used. Cells were fixed in 4% paraformaldehyde in PBS for 1 h at 4 °C. The cells were then washed overnight in PBS at 4 °C. Dehydration was performed in an ascending series of ethanol and embedding of the cells was performed in LR White. Sections (60 nm thick) were cut and placed on nickel grids (Sigma). The grids were floated on a drop of nonimmune rabbit serum (Dako) for 20 min and then transferred onto drops of a 1:100 dilution of a monoclonal anti-actin antibody (AC-40; Sigma). After incubation with primary antibodies for 30 min, the grids were rinsed in PBS. The grids were then exposed to biotinylated rabbit anti-mouse immunoglobulins (Dako) diluted 1:100 and then again washed in PBS. Afterwards, the grids were transferred onto drops of a 1:20 dilution of 10 nm gold particles conjugated to streptavidin (Sigma) and incubated for 30 min. The grids were then washed in PBS and dried. All incubation steps were performed at room temperature. Control specimens were incubated with nonimmune antiserum (normal mouse serum, Dako). The preparations were examined using a transmission electron microscope JEM 100 CX (JEOL, Japan) at 80 kV. Statistical analysis was performed by using Levene'a and Kruskala-Wallisa test. Calculations were carried out by using the computer program STATISTICA for Windows 5.1.

Results

To examine the reorganization of F-actin in K-562 and HL-60 cell lines, TRITC-phalloidin labeling was used. There were not only changes in the reorganization of F-actin but also in the morphology of the cells. Rearrangement of the actin system was dependent on the dose of taxol in both cell lines. We observed cells with disruption of F-actin microfilaments, but there were also cells with rather regular distribution throughout the cytoplasm. The concentration of F-actin as aggregates at the periphery, at the site of apoptotic body formation, was often observed (Fig. 1a–d). The translocation of F-actin from periphery to the centre of cells was also seen (Fig. 1 b,d). In a range of doses of 0.02–0.2 μM of taxol, cells showed distribution of F-actin similar to that of control cells. There was intense labeling of actin filaments in the vicinity to the surface of cells in both lines (Fig. 2 a-d). The treatment of K-562 with a range of concentrations of 2–10 μM of taxol resulted in a marked increase of F-actin labeling which was distributed throughout the cell. There were cells with increased labeling not only at the surface of the cell but also in the area of the nucleus. This localization was confirmed by confocal microscopy as seen in Figure 3. HL-60 cells treated with the range of concentrations of 2–10 μM of taxol showed not only a number of cells with a concentration of F-actin labeling distributed regularly in cytoplasm, but also at the site of apoptotic body formations (Fig. 4). There were shrinking cells with characteristic apoptotic morphology. Diagram 1 shows that the mean percentage of viable cells decreases significantly with the dose of taxol ($p<0.001$). Actin was also visualized at the ultrastructural level by using an immuno-gold technique. Immunogold labeling of actin was observed in control cells and cells treated with all doses of taxol in both cell lines. Labeling was localized in the nuclei and the cytoplasm. Positivity for actin in the nuclei of the cells treated with lower doses of taxol or control cells without taxol was localized in the vicinity of the nuclear envelope, probably in association with nuclear lamins (Fig. 5). There were also gold particles scattered throughout the nucleus, seemingly associated with the heterochromatin and around the nucleolus (Fig. 6). Significant changes in the morphology of the cells in both of the cell lines, but especially in the HL-60 cells, occurred after treatment with taxol in a range of concentrations between 2–10 μM. There was dilation of the endoplasmic reticulum and the nuclear envelope. In the nucleus, compaction and margination of chromatin with strong labeling for actin was observed (Fig. 7). Positive labeling for actin was not found in the controls treated with normal mouse serum instead of primary antibody (Fig. 8).

Discussion

The cytoskeleton is a promising target for therapeutic intervention and offers many opportunities in cancer treatment. Actin filaments may be considered as novel targets for cancer

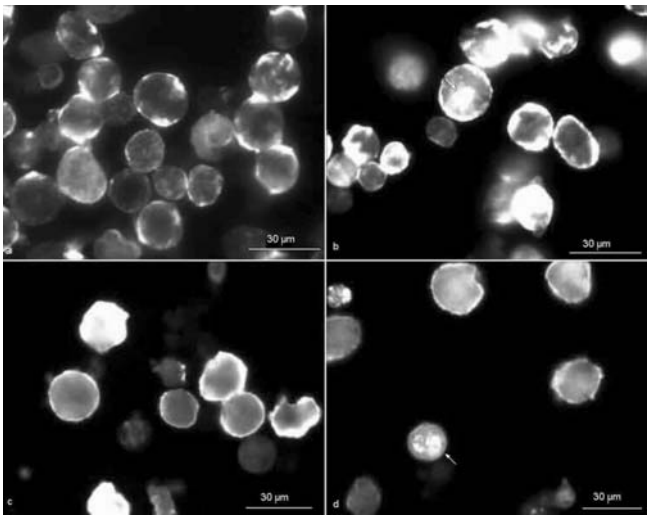

Figure 1. K-562 cells treated with 2 µM (a) and 10 µM (b) taxol. F-actin labeling is seen especially at the cell periphery and bright staining in the centre of the cell is also observed (arrow). HL-60 cells cultured in the presence of 2 µM (c) and 5 µM (d) of taxol. Labeling with rhodamine conjugated to phalloidin reveals F-actin regularly distributed throughout the cells with strong fluorescence at the surface of the cells. Strong

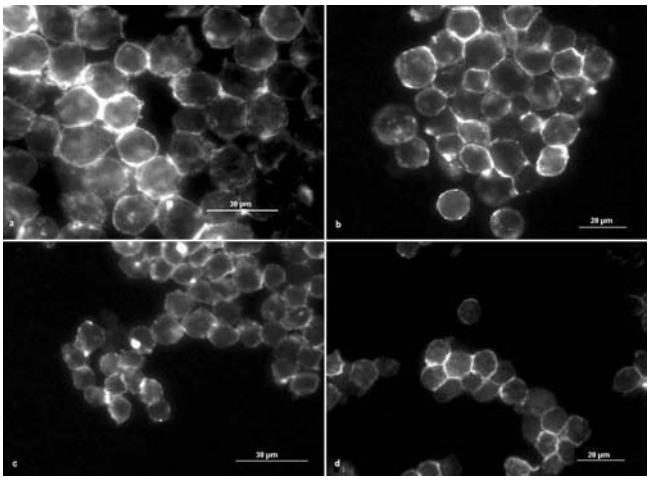

Figure 2. K-562 and HL-60 cells without taxol (a, c). K-562 cells (b) and HL-60 (d) cells treated with 0.02 µM taxol. Labeling for F-actin is present as a brightly fluorescent ring at the cell periphery. In the cytoplasm F-actin labeling in a weak punctuate pattern can be observed. Bar 30 µm (a,c); Bar 20 µm (b,d).

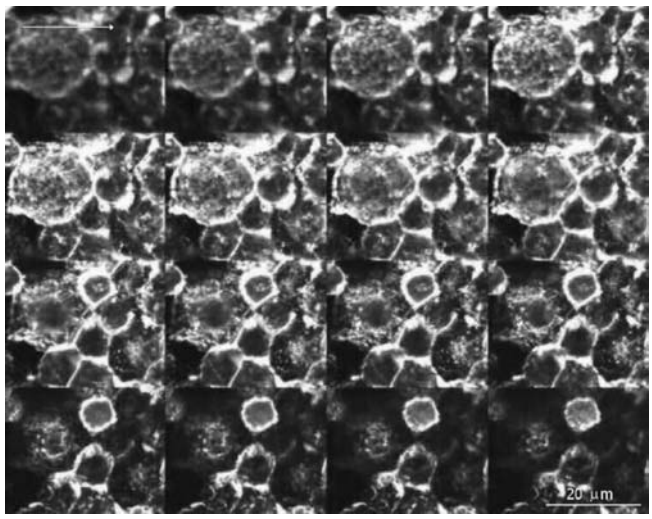

Figure 3. Z-scan series through the K-562 cell treated with 2 µM taxol with TRITC-phalloidin labeling for F-actin. The cell cortex is F-actin rich and in some sections labeling in the nucleus can be very clearly observed. Bar 20 µm.

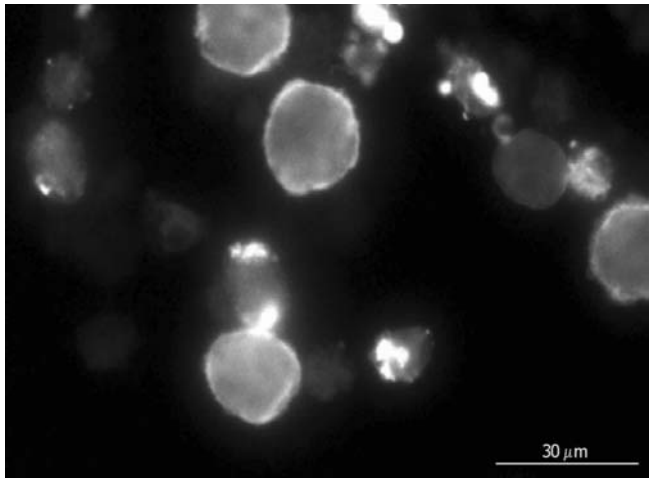

Figure 4. HL-60 cells cultured with 10 µM taxol labeled with rhodamine conjugated to phalloidin. Apart from cells with a regular distribution of F-actin labeling throughout, there are shrinking cells with a strong concentration of F-actin labeling as aggregates at their surface. Bar 30 µm.

therapy. There are a number of known inhibitors of microtubules, including taxol and colchicines which are used in cancer chemotherapy [36, 37] although currently, no actin-targeted inhibitors that are considered as anti-cancer drugs [36, 38] are used. It is well established that microtubule and actin filament networks are linked both physically and functionally. The present report and our previous studies [6, 7, 16, 35, 38–41] have tried to answer the question whether cytostatic drugs have an influence on the reorganization of all the main cytoskeletal proteins. In this report we have chosen taxol, as the well known stabilizer of microtubule polymers, which is a terpene compound obtained from the bark of *Taxus brevifolia*. It is characterized by a strong affinity to tubulin protein and a remarkable antitumor activity *in vitro* and *in vivo* [42]. Here, we address the issue to find whether a stabilizer of one system could have an effect on the organization of the other filament system. BLANCAFLOR [43] observed that taxol as a microtubule-stabilizing drug improved the quality

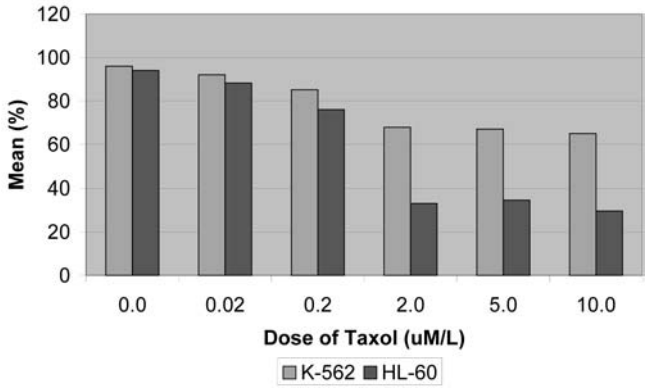

Diagram 1. The influence of taxol on mean percentage of viable cells.

of the F-actin system by stabilizing the cortical actin networks. Both systems are tightly coordinated by a number of protein which are implicated in the interaction between them [34]. This may suggest that they respond in a coordinated way to taxol although, the machinery responsible for the flow of information between actin and tubulin has not been fully elucidated. Here we showed that the activity of taxol was associated with the reorganization of F-actin dependent on its dose. There was intense labeling of actin filaments in the vicinity of the surface of cells treated with the range of doses of 0.02–02 μM of taxol in both cell lines. The treatment of K-562 cells with concentrations 2–10 μM of taxol resulted in a marked increase in F-actin labeling which was distributed regularly throughout the cytoplasm with especially intense labeling close to the surface of the cells. HL-60 cells which

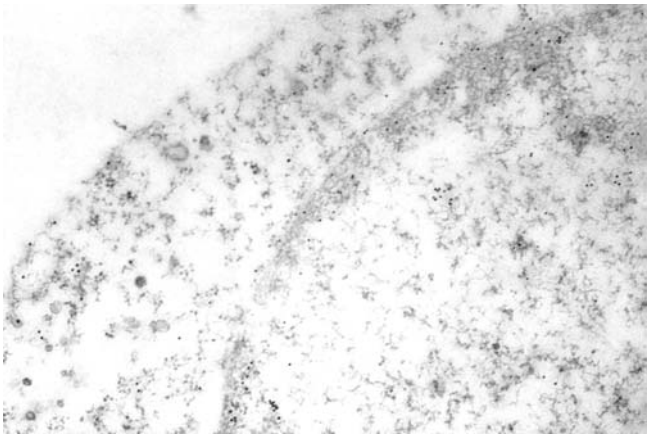

Figure 5. Electron micrograph of K-562 cell cultured in 0.2 μM taxol. Positivity for actin is present in the nucleus and in the cytoplasm. In the nucleus, gold particles are localized close to the nuclear envelope and scattered throughout the nucleus. Gold particles are particularly associated with heterochromatin. Magnification x 25 000.

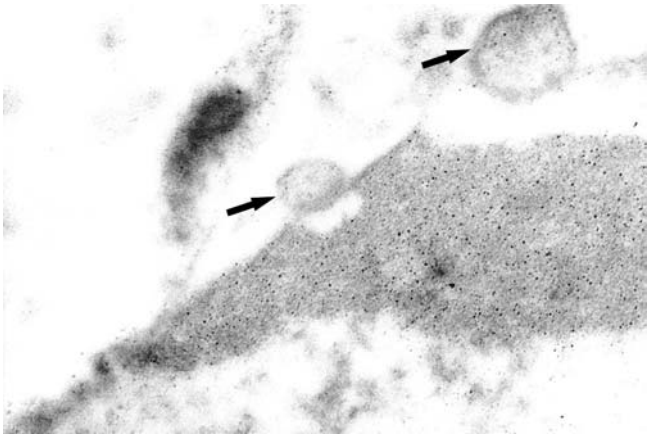

Figure 7. A part of nucleus of an apoptotic HL-60 cell treated with 10 μM taxol with gold particles demonstrating actin in the margination of nuclear chromatin and in nuclear fragments (arrows). Magnification x 39 000.

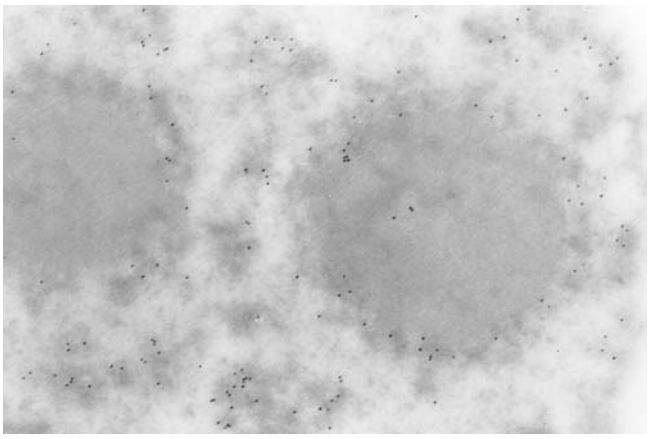

Figure 6. HL-60 cell from the culture with 0.2 μM taxol showing gold particles demonstrating actin around the nucleolus and associated with heterochromatin. Magnification x 40 000.

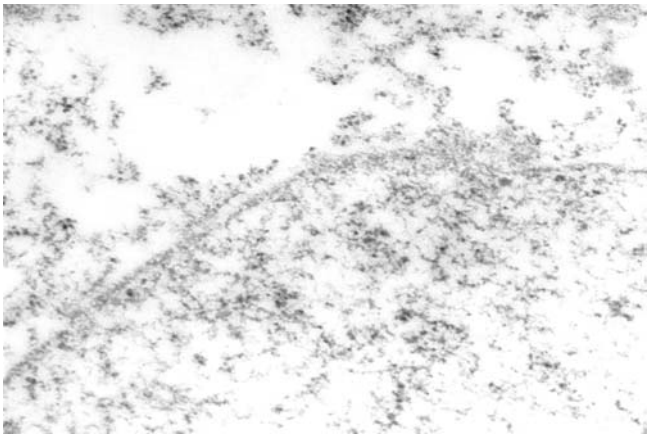

Figure 8. Electron micrograph of an HL-60 cell showing the control reaction with normal mouse serum instead of primary antibodies. Magnification x 34 000.

are more susceptible to apoptosis, showed a concentration of F-actin as aggregates at their periphery at the site of apoptotic bodies and translocation of F-actin from the periphery to the centre of cells. Immunogold labeling for actin was found to be associated with compaction and margination of nuclear chromatin in cells with morphological features of apoptosis, as has been shown in other studies [6, 7, 44]. GANGEMI et al [45] also observed the appearance of morphological features consistent with the process of apoptosis in HL-60 cells treated with taxol. However, a direct link between cytoskeletal disruption and subsequent apoptosis has not been established yet, although there is a great deal of evidence that actin filaments are cleaved during apoptosis [4, 8, 44, 46]. In the present study, we have focused on the actin reorganization after treatment with taxol, the active cancer chemotherapeutic agent that acts on cells by tubulin system. We have demonstrated a dose dependent reorganization of actin after treatment with taxol and also our observations at the ultrastructural level suggested actin involvement in chromatin reorganization during the process of apoptosis. Studies on the cytoskeleton suggest that the main cytoskeletal proteins may represent an important target for chemotherapy. The actin system is involved in cell motility and it seems to be an attractive target for the development of new anti-cancer drugs. The review by RAO and LI [47] showed that so far there have been no clinically proven drugs developed by targeting the intermediate filaments and microfilaments. Understanding of the rearrangement of the actin system in malignant cells and the effort to find drugs targeting specifically to actin may help to fight cancer in the future.

References

[1] ABERCROMBIE M. The crawling movement of metazoan cells. Proc R Soc Lond B Biol Sci 1980; 207: 129–147.

[2] CARLIER MF, RESSAD F, PANTALONI D. Control of actin dynamics in cell motility. Role of ADF/cofilin. J Biol Chem 1999; 274: 33827–33830.

[3] BANDO M, MIYAKE Y, SHIINA M, WACHI M, NAGAI K et al. Actin cytoskeleton is required for early apoptosis signaling induced by anti-Fas antibody but not Fas ligand in murine B lymphoma A20 cells. Biochem Biophys Res Commun 2002; 290: 268–274.

[4] BROWN SB, BAILEY K, SAVILL J. Actin is cleaved during constitutive apoptosis. Biochem J 1997; 323: 233–237.

[5] COTTER TG, LENNON SV, GLYNN JM, GREEN DR. Microfilament-disrupting agents prevent the formation of apoptotic bodies in tumor cells undergoing apoptosis. Cancer Res 1992; 52: 997–1005.

[6] GRZANKA A. Actin distribution patterns in HL-60 leukemia cells treated with etoposide. Acta Histochem 2001a; 103: 453–464.

[7] GRZANKA A, GRZANKA D, ORLIKOWSKA M. Cytoskeletal reorganization during process of apoptosis induced by cytostatic drugs in K-562 and HL-60 leukemia cell lines. Biochem Pharmacol 2003; 66: 1611–1617.

[8] LEVEE MG, DABROWSKA MI, LELLI JL, HINSHAW DB. Actin polymerization and depolymerization during apoptosis in HL-60 cells. Am J Physiol 1996; 271: C1981–C1992.

[9] VESELSKA R, ZITTERBART K, JELINKOVA S, NERADIL J, SVOBODA A. Specific cytoskeleton changes during apoptosis accompanying induced differentiation of HL-60 myeloid leukaemia cells. Oncology Rep 2003; 10: 1049–1058.

[10] WHITE SR, WILLIAMS P, WOJCIK KR, SUN S, HIEMSTRA PS et al. Initiation of apoptosis by actin cytoskeletal derangement in human airway epithelial cells. Am J Respir Cell Mol Biol 2001; 24: 282–294.

[11] RANDO OJ, ZHAO K, CRABTREE GR. Searching for a function for nuclear actin. Trends Cell Biol 2000; 10: 92–97.

[12] AMANKWAH KS, DE BONI U. Ultrastructural localization of filamentous actin within neuronal interphase nuclei in situ. Exp Cell Res 1994; 210: 315–325.

[13] BASSELL GJ, TANEJA KL, KISLAUSKIS EH, SUNDELL CL, POWERS CM et al. Actin filaments and the spatial positioning of mRNAS. Adv Exp Med Biol 1994; 358: 183–189.

[14] FUNAKI K, KATSUMOTO T, IINO A. Immunocytochemical localization of actin in the nucleolus of rat oocytes. Biol Cell 1995; 84: 139–146.

[15] MILANKOV K, DE BONI U. Cytochemical localization of actin and myosin aggregates in interphase nuclei in situ. Exp Cell Res 1993; 209: 189–199.

[16] NAKAYASU H, UEDA K. Association of actin with the nuclear matrix from bovine lymphocytes. Exp Cell Res 1983; 143: 55–62.

[17] PARFENOV VN, GALAKTIONOV KI. Intranuclear actin microfilaments in the oocytes of the common frog. Tsitologiia 1987; 29: 142–149.

[18] SAUMAN I, BERRY SJ. An actin infrastructure is associated with eukaryotic chromosomes: structural and functional significance. Eur J Cell Biol 1994; 64: 348–356.

[19] PERCIPALLE P, ZHAO J, POPE B, WEEDS A, LINDBERG U, DANEHOLT B. Actin bound to the heterogeneous nuclear ribonucleoprotein hrp36 is associated with Balbiani ring mRNA from the gene to polysomes. J Cell Biol 2001; 153: 229–236.

[20] PUCK KK, KRYSTOSEK A. Role of cytoskeleton in genome regulation and cancer. Int Rev Cytol 1992; 132: 75–108.

[21] ZHAO K, WANG W, RANDO OJ, XUE Y, SWIDEREK K et al. Rapid and phosphoinositol-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. Cell 1998; 95: 625–636.

[22] GONSIOR SM, PLATZ S, BUCHMEIER S, SCHEER U, JOCKUSCH BM, HINSSEN H. Conformational difference between nuclear and cytoplasmic actin as detected by a monoclonal antibody. J Cell Sci 1999; 112: 797–809.

[23] MANFREDI JJ, PARNESS J, HORWITZ SB. Taxol binds to cellular microtubules. J Cell Biol 1982; 94: 688–696.

[24] PARNESS J, HORWITZ SB. Taxol binds to polymerized tubulin in vitro. J Cell Biol 1981; 91: 479–487.

[25] SCHIFF PB, HORWITZ SB. Taxol stabilizes microtubules in mouse fibroblast cells. Proc Natl Acad Sci USA 1980; 77: 1561–1565.

[26] BLAGOSKLONNY MV, ROBEY R, SHEIKH MS, FOJO T. Paclitaxel-induced FasL-independent apoptosis and slow

(non-apoptotic) cell death. Cancer Biol Ther 2002; 1: 113–117.

[27] FAN W. Possible mechanisms of paclitaxel-induced apoptosis. Biochem Pharmacol 1999; 57: 1215–1221.

[28] IRELAND CM, PITTMAN SM. Tubulin alterations in taxol-induced apoptosis parallel those observed with other drugs. Biochem Pharmacol 1995; 49: 1491–1499.

[29] LONG BH, FAIRCHILD CR. Paclitaxel inhibits progression of mitotic cells to G1 phase by interference with spindle formation without affecting other microtubule functions during anaphase and telephase. Cancer Res 1994; 54: 4355–4361.

[30] BONNETERRE J, TUBIANA-HULIN M, CATIMEL G. Paclitaxel, epirubicin and cyclophosphamide combination in first-line treatment of metastatic breast cancer: a dose escalation study. Oncology 2004; 66: 185–191.

[31] EISENHAUER EA, VERMORKEN JB. The taxoids. Comparative clinical pharmacology and therapeutic potential. Drugs 1998; 55: 5–30.

[32] LIEBMANN JE, COOK JA, LIPSCHULTZ C, TEAGUE D, FISHER J, MITCHELL JB. Cytotoxic studies of paclitaxel (Taxol) in human tumour cell lines. Br J Cancer 1993; 68: 1104–1109.

[33] WISEMAN LR, SPENCER CM. Paclitaxel. An update of its use in the treatment of metastatic breast cancer and ovarian and other gynaecological cancers. Drugs Aging 1998; 12: 305–334.

[34] RODRIGUEZ OC, SCHAEFEWR AW, MANDATO CA, FORSCHER P BEMENT WM, WATERMAN-STORER CM. Conserved microtubule-actin interactions in cell movement and morphogenesis. Nat Cell Biol 2003; 5: 599–609.

[35] GRZANKA A, GRZANKA D. Distribution of actin in etoposide-induced human leukemia cell line K-562 using fluorescence and immunoelectron microscopy technique. Pol J Pathology 2002; 53: 43–50.

[36] JORDAN A, HADFIELD JA, LAWRENCE NJ, McGOWN AT. Tubulin as a target for anticancer drugs: agents which interact with the mitotic spindle. Med Res Rev 1998; 4: 259–296.

[37] SHI Q, CHEN K, MORRIS-NATSCHKE SL, LEE KH. Recent progress in the development of tubulin inhibitors as antimitotic antitumor agents. Curr Pharm Des 1998; 4: 219–248.

[38] JORDAN MA, WILSON L. Microtubules and actin filaments: dynamic targets for cancer chemotherapy. Curr Opin Cell Biol 1998; 10: 123–130.

[39] GRZANKA A. Estimation of changes in tubulin induced with etoposide in human leukemia cells line K-562 by immunofluorescence microscope. Neoplasma 2001b; 48: 48–51.

[40] GRZANKA A. Estimation of changes in vimentin filaments induced by etoposide and doxorubicin in human leukemia cell line K-562 by using immunofluorescence microscopy. Neoplasma 2001c; 48: 285–289.

[41] GRZANKA A, GRZANKA D. The influence of etoposide on the distribution of tubulin in human leukaemia cell line Hl-60. Med Sci Monit 2003; 9: BR66–9.

[42] ROWINSKY EK, CAZENNAVE LA, DONEHOWER RC. Taxol: a novel investigational antimicrotubule agent. J Natl Cancer Inst 1990; 82: 1247–1259.

[43] BLANCAFLOR EB. Cortical actin filaments potentially interact with cortical microtubules in regulating polarity of cell expansion in primary roots of maize (Zea mays L.). J Plant Growth Regul 2000; 19: 406–414.

[44] LU Y, JAMIESON L, BRASIER AR, FIELDS AP. NF-kappaB/RelA transactivation is required for atypical protein kinase C iota-mediated cell survival. Oncogene 2001; 20: 4777–4792.

[45] WAN YF, GUO XQ, WANG ZH, YING K, YAO MH. Effects of paclitaxel on proliferation and apoptosis in human acute myeloid leukemia HL-60 cells. Acta Pharmacol Sin 2004; 25: 378–384.

[46] GANGEMI RM, TISO M, MARCHETTI C, SEVERI AB, FABBI M. Taxol cytotoxicity on human leukemia cell lines is a function of their susceptibility to programmed cell death. Cancer Chemother Pharmacol 1995; 36: 385–392.

[47] RAO JY, LI N. Microfilament actin remodeling as a potential target for cancer drug development. Curr Cancer Drug Targets 2004; 4: 345–354.