Hepatocyte growth factor (HGF), heat shock proteins (HSPs) and multidrug resistance protein (MRP) expression in co-culture of colon tumor spheroids with normal cells after incubation with interleukin-1β (IL-1β) and/or camptothecin (CPT-11)

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Tumor chemoresistance and metastasis are some of the most important problems in colon cancer therapy. In the present study, co-cultures of human colon carcinoma cell spheroids, obtained from different grades of tumor, with human colon epithelium, myofibroblast and endothelial cell monolayers were performed. The purpose of these co-cultures was to reflect, in vitro conditions, different stages of colon tumor development. In order to investigate the invasive capacities of the tumor cells and their resistance to chemotherapy, HGF, HSP27, HSP72 and MRP levels were analyzed after incubation of the co-cultures with IL-1β and irinotecan (CPT-11) added as single agents or in combination. Myofibroblasts produced significantly higher amounts of HGF than epithelial cells. Tumor cells released trace amounts of this molecule. In co-cultures, IL-1β induced HGF release, while CPT-11 alone or combined with IL-1β decreased HGF secretion. An immunoblotting analysis followed by densitometry revealed that the combination of IL-1β plus CPT-11 added to the co-cultures led to a decrease in HSPs and MRP levels. In conclusion, direct and paracrine interactions of colon tumor cell spheroids with normal cells and exogenously added CPT-11 change HSP27, HSP72 and MRP expression in comparison to monocultures. IL-1β and CPT-11, dependent on whether they are added separately or jointly, differentially modulate HGF expression in monocultures of colon tumor spheroids or normal cells and their co-cultures.

Keywords: Co-culture, Colon carcinoma, Heat shock proteins, Hepatocyte growth factor, Multidrug resistance protein

Colon carcinoma (CRC) is one of the most common causes of deaths because the majority of patients are diagnosed at advanced stages of the disease. The major standard regimen in adjuvant and palliative chemotherapy of CRC is 5-fluorouracil (5-FU) commonly administered with leucovorin (LV). However, after failure with 5-FU/LV, additional use of irinotecan (camptothecin) (CPT-11) as a second-line treatment is advised. CPT-11 is also confirmed to be the most active when used as a single agent both in first- and second-line treatments1. Irinotecan is a synthetic derivative of a plant alkaloid which possesses a pronounced activity against colorectal cancer both in in vitro and in vivo models and in the clinical setting2,3. When hydrolyzed by carboxylesterase to an active metabolite, SN-38, this drug becomes a potent inhibitor of the nuclear enzyme topoisomerase I (topo I)3. The active form of CPT-11 generates a modification of DNA topology by leading to transient single strand breaks and, ultimately, to tumor cell apoptosis4,5. Unfortunately, a limiting factor for successful cancer chemotherapy is the occurrence of the multidrug resistance (MDR) phenotype6 i.e. simultaneous resistance to unrelated drugs7. Drug-resistance mechanisms are linked with decreased accumulation or increased efflux of cytotoxic drugs from cells and require over-expression of P-glycoproteins (Pgps) encoded by MDR genes and/or the multidrug resistance-associated protein (MRP)8. Both MRP and Pgp act as drug-efflux pumps, but Pgp has its greatest affinity for large hydrophobic cations whereas MRP is most

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effective in organic lipophilic anion transport\textsuperscript{9}. Although the mechanisms of both Pgp and MRP resistance concern tumors which are defective in drug accumulation, in colorectal cancers the MRP mechanism is more frequent than Pgp\textsuperscript{10,11}. Human MRP is a membrane integral 190 kDa protein which contributes to MDR influencing ATP-dependent absorption and distribution of various drugs, among others CPT-11, and active membrane transport for removal of those drugs from cells, thus affecting their efficacy and toxicity\textsuperscript{12,13}.

Cytoprotective effects in response to a wide variety of environmental insults including chemotherapy are also linked with the expression of heat shock proteins (HSPs). They are a group of constitutively expressed proteins, called molecular chaperones, involved in controlling protein folding, signaling, traffic, and degradation, and cytoskeleton organization, which can also be induced by different stressors such as heat, radiation, oxidative burst or cytotoxic drugs\textsuperscript{14-16}. Among the HSP family, HSP72 and HSP27 have been implicated in carcinogenesis and chemoresistance via several mechanisms protecting cells against apoptosis. These proteins can block cytochrome c release from mitochondria and inhibit the formation of apoptosome, procaspase 9 activation or phosphorylation of JNK kinase\textsuperscript{17}. On the other hand, HSPs have been shown to protect normal cells, e.g. surface colonocytes, against injury and to render mucosal epithelial cells less susceptible to ambient conditions allowing them to retain their function and viability under stress factors during chemotherapy\textsuperscript{18}. In colorectal carcinoma, the major lethal cause is metastasis. It has been revealed that HSPs, and especially HSP27, are abundantly expressed in CRC and play an important role in the progression and metastasis of this carcinoma\textsuperscript{19}. Beside the intrinsic mechanisms reducing intracellular drug accumulation, the tumor microenvironment also influences cell response to cytotoxic drugs\textsuperscript{20}. It has been found that over-expression of the hepatocyte growth factor (HGF) in the tumor stroma is associated with colon cancer progression and invasion\textsuperscript{21,22}. HGF also alters the response of cancer cells to cytotoxic agents including CPT-11. Recent findings suggest that HGF influences cell survival and protects tumor or normal epithelial cells against chemotherapeutic drugs by activating anti-apoptotic pathways and increasing DNA repair\textsuperscript{23,24}. Cancer cell progression and resistance to chemotherapeutic drugs strongly depend on direct tumor-stromal cell interactions. In turn, the secretory activity of stromal cells is up-regulated by soluble cytokines and growth factors secreted by tumor cells\textsuperscript{25}. IL-1β is one of the factors influencing the angiogenesis, progression and invasiveness of colon carcinoma. This pleiotropic cytokine is secreted by cells in the tumor stroma and to a lesser extent by the malignant cells themselves\textsuperscript{25}. Generally, over-expression of IL-1β in colon carcinomas is associated with a bad prognosis.

The present study has been designed to evaluate HSP27, HSP72 and MRP expression in co-cultures of colon carcinoma cell spheroids obtained from different stages of tumor with normal colonic epithelium, myofibroblast and endothelial cell monolayers after the addition of IL-1β and/or CPT-11. A second aim is to assess the expression of HGF in the analyzed co-culture conditions.

**Materials and Methods**

**Cell culture**—Human colon adenocarcinoma cell lines HT29 (ATCC No. HTB-38), derived from a grade I tumor, LS180 (ATCC No. CL-187), from a grade II tumor and SW948 (ATCC No. CCL-237), from a grade III tumor, were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) (Gibco\texttrademark, Paisley, UK) and antibiotics (100 U/ml penicillin and 100 \(\mu\)g/ml streptomycin) (Sigma, St. Louis, MO, USA) at 37\degree C in a humidified atmosphere with 5% CO\textsubscript{2}.

Human normal colon myofibroblasts CCD-18Co (ATCC No. CRL-1459) and normal epithelial cells CDD 841 CoTr (ATCC No. CRL-1807) were cultured in RPMI 1640 + DMEM (1:1) medium (Sigma) supplemented with 10% FCS at 37\degree C (CCD-18Co) or 34\degree C (CDD 841 CoTr) in a 5% CO\textsubscript{2}/95% air atmosphere.

Human normal umbilical vein vascular endothelium HUVEC (ATCC No. CRL-1730) was cultured in CS-C medium (Sigma) supplemented with 75 \(\mu\)g/ml endothelial cell growth factor (ECGF) (Sigma) and 10% FCS in a humidified atmosphere with 5% CO\textsubscript{2}.

**Preparation of tumor cell spheroids**—Tumor cell spheroids were prepared by the liquid overlay method, as described previously\textsuperscript{26}. In brief, the tumor cell suspension (200 µl) at a density of \(1\times10^5\) cells/ml in RPMI 1640 medium supplemented with 10% FCS was plated on 1% agarose-coated 96-multiwell culture plates (2\times10^4 cells/well). After 4 days’ incubation at...
37°C in a humidified atmosphere with 5% CO₂, the cells formed spheroids.

**Co-culture of tumor spheroids with normal cell monolayers**—The tumor spheroids were harvested with glass pipettes from the agarose-coated microplates and transferred into a Petri dish filled with warm RPMI 1640 medium. After 5 min washing, 5 spheroids each were transferred onto confluent myofibroblast or colon epithelium or HUVEC monolayers (1×10⁵ cells/ml) in 24-well tissue culture plates in RPMI 1640 medium supplemented with 2% FCS and incubated at 37°C in a humidified atmosphere with 5% CO₂. Parallel experiments were performed with tumor spheroids or normal cell monolayers alone as culture controls. After 12 h culture, supernatants and cell lysates were collected and stored at -80°C until further estimation.

Co-cultures of tumor spheroids with colon epithelial cells or myofibroblasts reflected the early stages of tumor development while interactions with endothelium resembled tumor cell dissemination via blood circulation.

**Exposure of cells to camptothecin (CPT-11) and IL-1β**—After 24 h incubation of the cells in RPMI 1640 with 10% FCS, the medium was discarded and fresh RPMI 1640 containing 2% FCS and CPT-11 (1 μg/ml) (MP Biomedicals, Inc., Eschwege, Germany) or/and IL-1β (2 ng/ml) (Strathmann Biotec GmbH) were added.

The incubation with the mentioned substances was performed for 6 h. Thereafter, the culture medium was changed, and the cells were incubated in a new one without the addition of CPT-11 and IL-1β for another 6 h at 37°C/5% CO₂. Culture supernatants and cell lysates were then collected and stored at -80°C no longer than 3 months.

**ELISA assay**—The level of human HGF was tested immunoenzymatically (ELISA) using a commercially available kit (R & D Systems, Minneapolis, MN, USA) according to the manufacturer’s instruction. The optical density at 450 nm with the correction wavelength 570 nm of each ELISA sample was determined using a microplate reader (Molecular Devices Corp., Emax, Menlo Park, CA, USA). Concentrations of HGF were calculated on the basis of a standard curve. The detection limit was less than 40 pg/ml.

**Indirect immunofluorescence**—Tumor and normal cells were inoculated in 8-well Lab-Tek slides at a density 1×10⁵ cells/ml in RPMI medium supplemented with 10% FCS. After 24 h the medium was changed to one containing 2% FCS, followed by the addition of IL-1β and CPT-11. Incubation was performed for the next 6 h. Thereafter, the cells were incubated in a new medium without CPT-11 and IL-1β for another 6 h. Then, the cells were washed twice with PBS, fixed in 4% paraformaldehyde for 10 min, washed twice with PBS, permeabilized with 0.1% Triton X-100 for 7 min, washed three times with PBS and blocked with 7.5% FCS for 1 h at room temperature. The cells were then incubated with primary goat polyclonal anti-HSP27, goat polyclonal anti-HSP72 or goat polyclonal anti-MRP antibodies (Santa Cruz Biotechnology, Inc.) overnight at 4°C. After three times washing with PBS, the cells were incubated with secondary FITC-conjugated donkey anti-goat IgG. The cells were examined with an Olympus BX51 System Microscope (Olympus Optical CO., LTD, Tokyo, Japan) and micrographs were prepared using AnalySIS® software (Soft Imaging System GmbH, Münster, Germany).

**Immunoblotting**—Total lysate of the treated cells was prepared by adding 150 μl of SDS-loading buffer with protease inhibitor cocktail (Sigma) directly to the cells cultured on 24-well plates and detaching them with a cell scraper. The protein concentration was determined using a BCA™ Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA).

The extracts were boiled for 5 min at 95°C and centrifuged at 10,000 rpm for 10 min at 4°C. Each protein sample (15 μl) containing exactly 100 μg of proteins was then loaded onto a 9% SDS-polyacrylamide gel. Following electrophoresis, the proteins were electrotransferred onto Immobilon-P transfer membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% skimmed milk for 1 h at room temperature and probed with primary goat anti-human IgG polyclonal antibodies: anti-HSP27, anti-HSP72 and anti-MRP (Santa Cruz Biotechnology, Inc.) for 2 h. After washing in PBS/1% Tween 20 (TBS-T), the membranes were labeled with alkaline phosphatase conjugated donkey anti-goat IgG-AP secondary antibodies (Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. After washing with TBS-T, the membranes were visualized with alkaline phosphatase substrates (5-bromo-4-chloro-3-indolyphosphate and nitroblue tetrazolium) (BCIP/NBT) (Sigma) in color development buffer (100 mM Tris with 5 mM Mg²⁺ at
pH 9.5). As an endogenous control to ensure the same protein loading for each sample β-actin was used.

**Densitometric analysis**—Semi-quantitative densitometric analysis of the bands was carried out with the Bio-Profil Bio-1D Windows Application V.99.03 program. The results were presented as density/volume of the bands.

**Statistical analysis**—Results are presented as means ± SD of three independent experiments. The data were analyzed using one-way analysis of variance ANOVA followed by Bonferroni’s multiple comparison post-hoc test. Differences of P≤0.001 (HGF analysis) were considered significant. Immunoblots after densitometry were calculated using Student’s t-test. P values lower than 0.05 were considered significant.

**Results**

In the present study, HSP27, HSP72 and MRP expression was analyzed in co-cultures of human colon tumor cell spheroids (HT29, LS180, SW948) with human normal colon epithelium (841CoTr), myofibroblast (18Co) and endothelial cell (HUVEC) monolayers. The level of these proteins was studied after incubation of the co-cultures with IL-1β (2 ng/ml) and/or CPT-11 (1 μg/ml). IL-1β and CPT-11 at the concentrations applied had no effect on tumor or normal cell viability during 6 h of incubation. The viability of the cells remained above 90% (data not shown). As an additional parameter, HGF production was analyzed in the described co-culture conditions.

**HGF level in co-cultures treated with IL-1β and/or CPT-11**—Tumor cells produced trace amounts of HGF (less than 81 pg/ml). Normal cells produced significantly higher amounts of HGF than tumor cells, with myofibroblasts (18Co) being the most active (8820.2 ± 294.9 pg/ml). In co-cultures, the HGF level decreased as compared to appropriate normal cell monocultures. The significant decreases were found in LS180 tumor spheroids co-cultured with 841CoTr cells and SW948 spheroids co-cultured with 18Co cells. An addition of IL-1β to the co-cultures resulted in a slight increase in HGF production. However, only one co-culture combination (HT29 + 18Co) was found to have a statistically significant increase. On the other hand, significant decreases were detected in HT29 and HUVEC monocultures (Fig. 1a). Similarly, CPT-11 significantly decreased the HGF level in tumor HT29 spheroid and 18Co and HUVEC normal

**Fig. 1**—HGF secretion in co-cultures of colon carcinoma cell spheroids with normal colon epithelial cells, myofibroblasts and endothelial cells during 6 h of incubation with IL-1β (a), CPT-11 (b), or IL-1β plus CPT-11 (c). ELISA test. Tumor cells produced trace amounts of HGF. Normal cells produced significantly higher amounts of HGF than tumor cells, with myofibroblasts (18Co) being the most active. In co-cultures, the HGF level decreased insignificantly. IL-1β increased HGF production, while CPT-11 used alone and in combination with IL-1β significantly limited HGF secretion [P values: *≤0.001: a co-culture of tumor/normal cells compared to an appropriate monoculture of normal cells; #≤0.001: a culture of tumor and/or normal cells treated with IL-1β and/or CPT-11 compared to an appropriate non-treated culture]
cell monocultures. The HGF level decreased significantly in HT29 spheroid co-cultures with colon epithelial cells, and similar decreases were observed in co-cultures of all the tested tumor cell spheroids with myofibroblasts. Moreover, CPT-11 in the co-culture of tumor spheroids and 18Co monocultures with the combination of IL-1β plus CPT-11, the HGF level significantly decreased. Moreover, in the tumor spheroid co-culture with 18Co cells, a significant decrease in the HGF level was found (Fig. 1c).

Localization of HSP27, HSP72 and MRP in normal and tumor cells—HSP27 and HSP72 were distributed uniformly in the cytoplasm of both tumor and normal cells as revealed by indirect immunofluorescence (Figs 2a-b and 3a-b, respectively). MRP is a transmembrane protein functioning as energy-dependent drug efflux pomp. As observed under fluorescence microscopy, immunoreactivity for MRP was mainly localized in the nuclear membrane of both tumor and normal cells (Fig. 4a-b). Interestingly, MRP accumulated especially at a pole of the nuclear membrane (see arrow in Fig. 4a). This may be associated with the local distribution of the granular endoplasmic reticulum.

Expression of HSP27, HSP72 and MRP in cancer and normal cells and their co-cultures after treatment with IL-1β and/or CPT-11—An analysis of the expression of HSP27, HSP72 and MRP was performed using the immunoblotting method followed by densitometry. Figures 5-7 show selected, representative immunoblots of three repeats and Fig. 8a-b and Fig. 9a-c represent the densitometric analysis. In endothelial cells, no HSP72 expression was detected. IL-1β and CPT-11+IL-1β significantly limited MRP and HSP27 expression in HT29 tumor cells, respectively. On the other hand, HSP27 and HSP72 levels significantly increased when SW948 tumor cell spheroids were incubated with CPT-11. In the normal 841CoTr cell culture, significant decreases in HSP72 after incubation with CPT-11 and CPT-11+IL-1β were detected.

In co-cultures of HT29 and SW948 tumor spheroids with 841CoTr normal cells, HSP27 and HSP72 expression significantly increased after the addition of CPT-11. The other co-cultures did not show significant changes in HSP27, HSP72 or MRP expression after incubation with IL-1β and/or CPT-11.

Discussion

The incidence of colorectal cancer (CRC) is dramatically increasing worldwide. What makes matters worse is that the majority of the patients are diagnosed at advanced stages of cancer, which are characterized by a high propensity to metastatization. It has already been shown that at the initial diagnosis disseminated, secondary tumors are detected in about 30-40% of CRC patients. Therefore, the use of adjuvant chemotherapy after primary operation of CRC is required. However, an important limiting factor for successful cancer treatment is the occurrence of the inherent multidrug resistance (MDR) phenotype. This phenomenon may exist intrinsically before cancer treatment or may be acquired due to environmental stress factors, e.g. chemotherapeutic agents. The existence of intrinsic drug resistance is clinically confirmed by relatively low response rates (<50%) of colon malignancies to chemotherapy. This is closely linked with the expression of genes encoding two membrane integral proteins that confer MDR. In colorectal carcinoma studies, P-glycoprotein (Pgp) and multidrug resistance-associated protein (MRP) have received most attention. These two proteins contribute to MDR by ATP-dependent active membrane transport removing extracellular cytotoxic drugs (ATP-dependent drug efflux pomp). Drug resistance is also associated with over-expression of heat shock proteins (HSPs) which protect cells from adverse environmental stresses. They prevent protein aggregation, promote refolding of denaturated proteins, mediate cell growth and, most importantly for chemoresistance, prevent apoptosis. Among the HSP family, HSP27 and HSP72 have been implicated in colon carcinoma cell resistance to chemotherapeutic agents. These proteins have also been shown to play an important role in the progression of CRC. It is evident that tumor development and response to chemotherapy strongly depend on the tumor microenvironment, i.e. the local cytokine and growth factor network and direct tumor-stromal cell interactions. In general, the interplays of tumor cells with the surrounding normal stroma are a primary focus for the study of tumor development. Organ specificity of the metastatic process in colon cancer suggests the importance of paracrine factors,
Fig. 2—Indirect immunofluorescence showing HSP27 distribution in normal endothelial cells (HUVEC) (a) and colon tumor cells (HT29) (b). Note the uniform localization of the protein in the cellular cytoplasm. 400×. Bar 20 μm.

Fig. 3—Indirect immunofluorescence showing HSP72 distribution in normal endothelial cells (HUVEC) (a) and colon tumor cells (HT29) (b). HSP72 is localized uniformly in the cytoplasm. 400×. Bar 20 μm.

Fig. 4—Indirect immunofluorescence localizing MRP in normal endothelial cells (HUVEC) (a) and colon tumor cells (HT29) (b). Note the distribution of MRP in the nuclear membrane. Magnification 400×. Bar 20 μm.
Fig. 5—Western blot analysis of HSP27, HSP72 and MRP after 6 h of incubation with IL-1β and/or CPT-11 in HT29 colon carcinoma cells. HSP27 and MRP insignificantly decreased while HSP72 increased after the addition of IL-1β alone and IL-1β plus CPT-11 to the culture. CPT-11 added alone elevated the level of the proteins.

Fig. 6—Western blot analysis of HSP27, HSP72 and MRP after 6 h of incubation with IL-1β and/or CPT-11 in normal colon epithelial cells (841 CoTr). The addition of IL-1β led to an increase in the HSP27 and a decrease in the HSP72 and the MRP levels. The level of HSPs and MRP slightly decreased after treatment with CPT-11 alone and IL-1β plus CPT-11.

Fig. 7—Western blot analysis of HSP27, HSP72 and MRP expression after 6 h of incubation with IL-1β and/or CPT-11 in the co-culture of human colon carcinoma cell spheroids (HT29) with human normal colon epithelial cells (841 CoTr). After treatment of the co-culture with IL-1β or CPT-11, the levels of the analyzed proteins increased. However, the protein levels decreased after the addition of IL-1β with CPT-11 to the co-culture as calculated after semiquantitative densitometry.

Among which hepatocyte growth factor (HGF) is the most potent regulator of tumor cell dissemination. Moreover, HGF is implicated in chemosensitivity of tumor cells in response to many clinically applied chemotherapeutic agents. Therefore, analysis of this factor is very important in evaluating the development of many cancers including colon carcinoma.

In the present study, myofibroblasts expressed high levels of HGF. This is in accordance with the commonly known fact that HGF is secreted mainly by stromal tissue including fibroblasts. Moreover, tumor cells up-regulate HGF secretion by mesenchymal tissue through the action of soluble cytokines and growth factors. One of these cytokines is IL-1β, which was demonstrated to be closely linked with the up-regulation of HGF production in tumor/normal cell co-cultures mainly by modulating the HGF expression in fibroblasts. It is also commonly known that cancers which over-express this cytokine have general bad prognoses. IL-1β is also a known pro-inflammatory factor influencing tumor cell development and migration. However, HGF, unlike e.g. IL-1β or VEGF exerts anti-inflammatory effects. Therefore, increased HGF level after IL-1β addition to the co-cultures may be a protective activity of normal cells against inflammatory process development and cancer progression. In turn, CPT-11 causes cell-cycle arrest and apoptosis and is not as potent factor inducing inflammation as, e.g. IL-1β. The use of CPT-11 was more appropriate to investigate tumor chemoresistance, whereas IL-1β represents factors influencing tumor dissemination. They act at different molecular pathways and therefore different influence on HGF expression in co-cultures of tumor/normal cells could be observed. However, the exact mechanisms explaining these processes and different activity of IL-1β and its combination with CPT-11 on HGF expression in tumor/normal co-cultures are currently in progress.

In the present study, the expression of MRP and HSP (HSP27 and HSP72) proteins was also analysed in mono- and co-cultures of colon carcinoma cell spheroids with normal cells. HSP production by tumor cells has been associated with cell resistance to chemotherapeutic agents. CPT-11 was used as an effective drug in the treatment of patients with advanced and metastatic colorectal cancer. This molecule targets the eucariotic nuclear DNA enzyme topoisomerase I (topo I). However, as one of the side effects, it induces tumor cell resistance by over-
Fig. 8—Semiquantitative results of densitometric analysis of the bands from the monocultures of tumor cell spheroids (a) and normal cells (b) are shown. $P<0.05$ – co-cultures treated with IL-1β, CPT-11 or their combination compared to untreated co-culture.
expression of HSPs and MRP\textsuperscript{39}. Additionally, it was confirmed that CPT-11 significantly induced HSP27 and HSP72 over-expression in advanced (SW948) colon cancer cell spheroids and their co-cultures with normal colonic epithelial cells. The high expression of HSPs and the occurrence of the multidrug resistance phenotype are closely linked with poor prognosis and progression of colorectal cancer and, in general, represent a limiting factor for successful cancer chemotherapy\textsuperscript{6,19,30,40}. It has, however, been shown that tumor cell resistance in numerous tissues is significantly altered during an inflammatory response. An acute inflammation is known to be a result of the induction of, among others, several pro-inflammatory
cytokines\(^1\). One of these cytokines is IL-1\(\beta\) which regulates the proliferation and differentiation of normal and malignant cells\(^2\). This cytokine is abundantly expressed at tumor sites where it influences not only the invasiveness of cancer cells but also may induce anti-tumor immunity\(^25\).

In conclusion, it is shown that IL-1\(\beta\) and CPT-11 influence HGF levels. The effect was dependent on the culture conditions (tumor and normal cell monocultures or their co-culture), tumor cell stage and whether IL-1\(\beta\) and CPT-11 were used alone or in combination. IL-1\(\beta\) and/or CPT-11 also influenced on HSP27, HSP72 and MRP production in tumor and normal cells.

References
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