12

The Impact of Extracellular Low pH on the Anti-Tumor Efficacy Against Mesothelioma

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1. Introduction

Inflammation and tumors have been demonstrated to have several common characteristics in their microenvironments. Extracellular acidosis is frequently associated both with inflammation area and tumor growth. Measurements of pH in peripheral tissues during the development of inflammation have shown extracellular pH values as low as 5.5–7.0 while the pH values of normal tissues are usually maintained at pH 7.4-7.5 mainly via pulmonary respiration and kidney perfusion of protons (Edlow & Sheldon, 1971). Similary, the extracellular pH in the central regions of tumors decreases below 6.7 in several tumors as a consequence of lactate accumulation derived from a lack of sufficient vascularization or an increase in tumor specific glycolysis under aerobic conditions combined with impaired mitochondrial oxidative phosphorylation (Simmen, 1993; Vaupel, 1989; Warburg, 1956). These pH declines affect cellular or tissue functions because their features are determined mainly by a variety of enzymatic proteins, and all enzymatic activities have each optimal pH. We have previously reported that the low pH conditions alter signal transductions. (1) The phosphorylations of several proteins were upregulated at low pH in leukemia cells (Fukamachi et al., 2001; Hirara et al., 2008). (2) CTIB, an Ikappa B beta variant, regulates cellular survival and gene expression exclusively under acidic environments in Chinese Hamster Ovary cells (Lao et al., 2005, 2006). Furthermore, the gene expressions related with tumor malignancy were upregulated at low pH in several tumor cell lines (Rofstad et al., 2006). These different characteristics dependent on extracellular pH provided us with a perspective that the inhibitory effect of anti-tumor drugs or molecular targeted inhibitors would vary at tumor-specific low pH, and the development of anti-tumor medicines, which have medical properties especially in acidic conditions, would lead to curative therapies for cancers.

Malignant mesothelioma is an aggressive tumor developed from the pleura or other mesothelioma surface. No efficient method for treatment, including chemotherapy and radiotherapy, has yet been established for advanced stage mesothelioma (Zucali & Giaccone, 2006). However, the efficacies of anti-tumor agents against not only mesothelioma but other tumors under acidic conditions have not yet been investigated exhaustively. Only some attributive information has emphasized the possibility of alteration in anti-tumor efficacy by extracellular pH and other conditions. The cytotoxicity of mitoxantrone and

topotecan was reduced at low extracellular pH in murine EMT6 and in human MGH-U1 cells (Vukovic & Tannock, 1977). The impaired efficacy of mitoxantrone by acidosis was confirmed in M1R rat mammary carcinoma cells (Jähde et al., 1990). MCF-7 human breast cancer cells in vitro were more susceptible to doxorubicin toxicity at pH 7.4 compared to pH 6.8, probably due to its weak base conformation (Raghunand et al., 1999). On the other hands, mitomycin C showed higher cytotoxicity at low pH condition against EMT6 tumor cells (Rockwell, 1986). The research on pH dependent inhibitory effects has accumulated gradually, however the exhaustive investigation of efficacy at low pH using anti-tumor agents and molecular targeted inhibitors against mesothelioma is urgent and important for combinational therapy of anti-tumor agents or development of new drugs.

The screening of molecular targeted inhibitors, which inhibit cell growth preferentially at low pH conditions, has the potential to produce new therapeutic agents with less adverse effect against normal tissues because the development of malignant mesothelioma is associated with inflammation derived from asbestos exposure and the insides of mesothelioma tissues are also acidificated due to the mechanisms described above (Jähde et al., 1992). In order to develop a new therapeutic modality, in this article we discuss the effects of tumor specific microenvironments, especially under low pH conditions, on the efficacy of classical anti-tumor drugs and molecular targeted inhibitors.

2. The SCADs inhibitor kits and experimental procedure

We previously compared the cytotoxic efficacies of 93 molecular targeted inhibitors in SCADs inhibitor kit 1 at pH 7.5 and pH 6.7 against HeLa cells (Fukamachi et al., 2010). The tumor characteristics, however, differ widely and, moreover, SCADs inhibitor kits 2 and 3 have a number of other inhibitors, so we examined the cytotoxic efficacies of 272 kinds of molecular targeted inhibitors using SCADs inhibitor kits 1, 2 and 3 under different pH conditions against mesothelioma cells.

The inhibitory effects of chemical compounds in the SCADs inhibitor kits at different pH conditions were estimated by WST assay, a modified procedure of the MTT assay, as described in our previous reports (Fukamachi et al., 2010). Human pleural mesothelioma cell line NCI-H2052 was cultured in RPMI-1640 medium. To maintain medium pH for the comparison of inhibitory effects under different pH conditions, our group has added Good's buffer to media instead of sodium bicarbonate and found that all tumor cell lines we tested can proliferated both at pH 7.5 and 6.7 without sodium bicarbonate although the proliferation at pH 6.7 was slower than that at pH 7.5. The alteration of medium pH was not significant at pH 6.7 whereas the pH of alkaline medium declined to 7.4 after proliferation for 5 days (Fukamachi et al., 2001; Lao et al., 2005, 2006).

3. The effect of extracellular pH on classical anti-tumor agents against mesothelioma

SCADs inhibitor kits include 17 anti-tumor medicines those have already been prescribed for treatment of cancers. As shown in Table 1, none of the classical anti-tumor agents achieved better results against mesothelioma under acidic conditions. Cisplatin, mitomycin C, daunorubicin, aclarubicin, vinblastine sulfate, doxorubicin and cytochalasin D showed less cytotoxicity under acidic conditions. No pH dependency was seen with

bleomycin sulfate, paclitaxel, actinomycin D, camptothecin or etoposide (Table 1, Fig.1). The remaining 6 medicines did not reduce cellular survival independently from medium pH at 2 microM.

Cytotoxicity at different pH	
higher cytotoxicity at low pH	–
lower cytotoxicity at low pH	Cisplatin, Mitomycin C, Daunorubicin/HCl, Doxorubicin/HCl Vinblastine sulfate, Aclarubicin, Cytochalasin D
no different cytotoxicity between pH 6.7 and 7.5	Bleomycin sulfate, Paclitaxel, Actinomycin D, Camptothecin Etoposide
no cytotoxicity under 2 micro M	5-FU, Bestatin, Methotrexate, Flutamide, Tamoxifen/citrate

Table 1. Cytotoxicity of classical anti-tumor drug at different pH conditions.

3.1 Cisplatin

The cytotoxicity of cisplatin has been reported previously to show high sensitivity at low pH in EMT mouse tumor or leukemia (Laurencot & Kennedy, 1995). However, the cytotoxicity against NCI-H2052 mesothelioma was impaired at pH 6.7 as shown in Table.1 and Fig.1. We suppose that this difference was derived from the alteration of intracellular pH value dependent on extracellular pH value and the composition of nutrients. In previous experiments, the medium pH values were set at pH 6.0 as acidic conditions in contrast to the pH value 6.7 in our reports. We confirmed that the intracellular pH values are maintained at pH above 7.1 in cells incubated in the medium with 10% FBS at pH 6.7 (data not shown). In addition to our experiments, the maintenance of intracellular pH values in weak acidic conditions was again confirmed in other tumor cell lines (Gerweck & Seethaaraman, 1996; Owen et al., 1997). Extracellular pH lower than 6.5, however, markedly reduced intracellular pH values (Rockwells, 1986). This large reduction of intracellular pH values has been associated with apoptosis being independent of the presence of anti-tumor agents, so the enhanced effect of cisplatin under acidic conditions below pH 6.0 might be derived from another mechanism. Although the pH sensitive, anti-tumor bis (aminoalcohol) dichloroplatinum(II) has been developed, but its low pH dependent cytotoxicity appeared pH only below pH 6.0 (Zorbas-Seifriends et al., 2006). The extracellular pH values in mesothelioma, are usually over 6.7 (Jahde et al., 1992), so we presume that the cytotoxic potential of cisplatin is reduced around at pH 6.7 in mesothelioma.

The mechanism of resistance against cisplatin under low pH conditions is unknown. Cisplatin-resistance has been related with the activity of several proteins such as BRCA1, which plays multiple role in DNA repair, or p21WAF1/CIP1 proteins (Alli et al., 2011; Wei et al., 2010). Although the activity of these proteins at pH 6.7 has not been investigated, acidosis affects several protein activity (Lao et al., 2006; Rofstad et al., 2006). So the alteration of these proteins' activity may contribute to the Cisplatin-resistance at low pH.

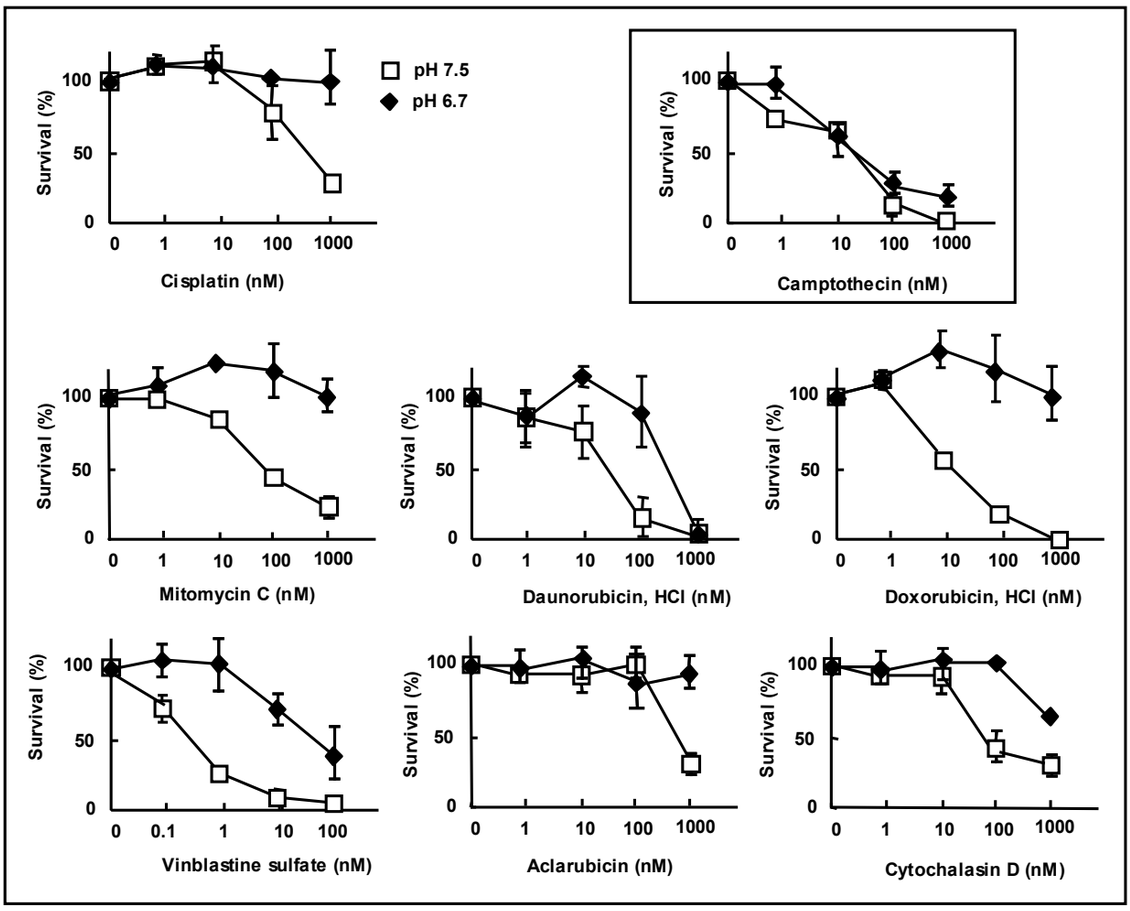

Fig. 1. pH-dependent cytotoxicity of classical anti-tumor drug.

3.2 Mitomycin C

Mitomycin C was reported to be potently its cytotoxic at low pH conditions against EMT6 tumor cells and this was associated with the upregulated binding of mitomycin C to DNA at low pH conditions (Rockwells, 1986). This result is different from our results, as shown in Table.1 and Fig.1. The medium pH values were at pH 5.7 in previous experiments, so probably the cytotoxicity of mitomycin C would be reduced at around pH 6.7. Resistance to mitomycin C by several cancers has been reported and related with glutathion S-transferase activity (Ruiz-Gomez et al., 2000). So the quantification of the expression or activity of glutathion S-transferase under acidic conditions may be important. Although the treatment against mesothelioma using mitomycin C has been investigated, its efficacy was less than those of cisplatin (Fennell et al., 2007) or oxalipalatin (Routh et al., 2011). In contrast, Co-treatment with mitomycin C, vinblastin and cisplatin achieved the improvement of symptoms including cough, dyspnoea and pains (Andreopoulou et al., 2004). So treatment with mitomycin C might be limited to improving the quality of life.

3.3 Doxorubicin and daunorubicin

The therapeutic effects of doxorubicin and daunorubicin against mesothelioma have been linked with the tumor-resistance mechanism of mesothelioma. Single treatment with doxorubicin and daunorubicin showed no significant anti-tumor activity (Harvey et al.,1984; Steele et al., 2001). Although this combination with cisplatin achieved some slight improvement, a high dose of doxorubicin was needed and this showed a toxic rather than curative effect (Stewart et al., 1994). These impaired efficacies of doxorubicin in mesothelioma have been thought to be due to upregulation of p-glycoprotein mediated drug efflux (Isobe et al., 1994) or multi drug associated protein (Kato et al., 1998). The expression of p-glycoprotein was up-regulated at low pH conditions in EMT6 cells and prostate cancer cells (Thews et al., 2006). This upregulation of p-glycoprotein is partially dependent on HIF-1 (Riganti et al., 2008) and extracellular low pH has been reported to induce HIF-1 (Mekhall et al., 2004). So, this mechanism might contribute to the pH-dependent loss of cytotoxicity as shown in Fig.1, in addition to the possibility derived from its weakly basic conformation (Raghunand et al., 1999). Drug resistance is also influenced by the activity of ROS scavengers. Most mesotheliomas express relatively large amount of manganese superoxide dismutase, catalase and cell surface NADH oxidase, and the cytotoxicitic function of ROS derived from doxorubicin were impaired in mesothelioma (Hedges et al., 2003; Kahlos et al., 1998).

3.4 Vinblastine sulfate, aclarubicin and cytochalasin D

These three agents has not been investigated at low pH conditions yet. So our experiment shown in Fig.1 was the first to show less cytotoxicity under acidic conditions. The reduced cytotoxicity of these three drugs, however, might be explained by the pH-dependent upregulation of p-glycoprotein as well as doxorubicin because vinblastine is also a p-glycoprotein substance (Gertner et al., 1998). Recent data indicated that the use of vinblastine should be limited to the improvement of symptoms as described above for mitomycin C (Andreopuoulou et al., 2004). It has been reported that the anti-tumor activity of aclarubicin is related to the production of ROS (Rogalska et al., 2008). So the mesothelioma cell, which expresses elevated amounts of ROS scavengers, may be resistant to aclarubicin.

The effects of cytochalasin D are derived from the breaking of actin filaments (Schiliwa, 1982). Human actin-depolymerizing factor and cofilin have been reported to be pH-sensitive (Pope et al., 2004). So this mechanism is likely to be associated with our results shown in Fig.1.

3.5 The pH dependency of other anti-tumor agents

Bleomycin was reported to show pH dependency in restricted conditions. The combination of high temperature and low pH enhanced the efficacy of bleomycin and 1,3-bis(2-chloroethyl)-1-nitrosourea but not methotrexate against Chinese hamster ovary, while there was no effect of pH at normal tissue temperature (Hahn & Shiu, 1983). The efficiency of bleomycin at low pH and high temperature has not been estimated against mesothelioma yet in vitro and vivo. Hyperthermia with bleomycin, however, may be worthwhile to examine against mesothelioma.

Although 5-Fluorouracil (5-FU) did not show cytotoxicity under both pH conditions as shown in Fig.1, high dose 5-FU previously showed the potential for pH dependent mesothelioma suppression (Nissen & Tanneberg, 1981). 5-FU inhibited the 3H-uridine incorporation preferentially at pH 7.4 compared to that at pH 6.8 at concentrations above 100 microM, so the comparison at each pH with higher doses of 5-FU is needed.

4. The effect of extracellular pH on molecular targeted inhibitors

As shown in Table 2, only four molecular targeted inhibitors, lovastatin, manumycin A, FTI-276 and cantharidin, showed higher cytotoxicity at low pH conditions in our exhaustively investigation against mesothelioma at different pH conditions using SCADs inhibitor kit. The inhibitory effect of aphidicolin, bisindolymaleimide I, N1,N12-diethylspermine, PKR inhibitor were impaired at low pH (Table 2, Fig.2).

Cytotoxicity at different pH	
higher cytotoxicity at low pH	Manumycin A, FTI-276, Cantharidin, Lovastatin
lower cytotoxicity at low pH	Aphidicolin, Bisindolymaleimide I/HCl, N1,N12-Diethylspermine PKR inhibitor
no different cytotoxicity between pH 6.7 and 7.5	Scriptaid, Trichostatin A, Cycloheximide, Radicicol, 17-AAG, Cucurbitacin PD 98059, MG-132, Lactacystin, Oligomycin, Bafilomycin A1, SB 225002 Monensin, Ouabain, Sanguinarine, Valinomycin, Nigericin, A23187 Ionomycin, Thapsigargin, Rotenone, Leptomycin B*, LY 83583, Chetomin α-Amanitin, MST-312, Akt Inhibitor IV, ATM kinase inhibitor Cdk2/9 inhibitor, AGL 2263, IKK-2 inhibitor VI, JAK Inhibitor I, PP2 SU11652, PDGF receptor tyrosine kinase inhibitor IV, LY-294002 Wortmannin, Go7874, KT5823, ZM 336372, SU1498 VEGFR receptor tyrosine kinase inhibitor III VEGF recptor 2 kinase inhibitor

Table 2. Cytotoxicity of molecular targeted inhibitor at different pH conditions.

4.1 Statin

4.1.1 The therapeutic capacity of statins against cancers

The pH-dependent cytotoxicity of lovastatin was observed previously in HeLa cells, mesothelioma cell line H2452 cells, pancreatic tumor cell line, BxPC-3 and Panc-1. Moreover, simvastatin, another lipophilic statin, inhibited cellular survival of HeLa cells as well as lovastatin (Fukamachi et al., 2010). These results indicated that pH-dependent cytotoxicity of lovastatin is common to several tumors and that other statins have similar properties. Statins (3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) block the *de novo* synthesis of cholesterol, resulting in lower plasma cholesterol levels. Although myopathy or

its more severe form rhabdomyolysis is a significant adverse effect of statin treatment, the incidence is typically less than 0.1% (Ballantyne et al., 2003). Other adverse effects including hyperplasia of the liver, squamous epithelial hyperplasia of cataracts and vascular lesions in the central nervous system have lower incidences or need extreme inhibition of the enzyme with high doses of statins (Gerson et al., 1989). Due to its active effect against cholesterol synthesis and relatively safe features, lovastatin and other statins have been prescribed for treatment of hypercholesterolaemia since the late 1980s (Tobert, 2003). Furthermore, statins might have potential for other coronary artery diseases. In patients without established cardiovascular disease but with cardiovascular risk factors, statin use was associated with significantly improved survival and large reductions in the risk of major cardiovascular events (Bruqts et al., 2009).

In addition to its great curative effect for vascular disease, the relationship of statin with cancer treatments has been discussed, including mesothelioma. Lovastatin inhibited the growth of T cell leukemia (Newman et al., 1994), pancreatic tumor (Muller et al., 1998), glioma (Jones et al., 1994), lung cancer (Maksimova et al., 2008) and prostate cancer (Hoque et al., 2008). Other statins as well as lovastatin showed cytotoxicity against tumor cell lines. Simvastatin against HT29 human colon cancer cells (Cho et al., 2008), glioma (Wu et al., 2009), pravastatin against hepatoma (Kawata et al., 1992) have been reported. Moreover, several statins reduced the migration of human pancreatic tumors and mouse melanoma (Kusama et al., 2002). In addition to the cytotoxicity and suppression of invasion against tumor cell lines, statins' curative effect against several cancers was confirmed in patients. Conventional chemotherapy using simvastatin with irinotecan, 5-FU and leucovorin (FOLFIRI) was a feasible regimen with promising anti-tumor activity against metastatic colorectal cancers (Lee et al., 2009). Fluvastatin reduces proliferation and increases apoptosis in women with high grade breast cancer (Garwood et al., 2010). These results indicated that statins inhibit tumor proliferation and have the potential to be applied for cancer treatments.

4.1.2 The mechanism of statins cytotoxicity; cholesterol and protein prenylation

The mechanism underlying statins' pH-dependent cytotoxicity is unclear. Statins inhibit HMG-CoA reductase that converts HMG-CoA to mevalonate, resulting in the reduction of farnesyl pyrophosphate, a substance of cholesterol. The important feature of cholesterol is that cholesterol is critical substance in plasma and vesicle membranes, so the inhibition of cholesterol synthesis by statins reduces mitochondrial membrane potential and induces the release of pro-apoptotic factors including cytochrome c, resulting in the apoptosis or the increase of doxorubicin sensitivity in hepatocellular carcinoma (Montero et al., 2008). The amount of cholesterol also contributed to the tumor cell migration (Sekine et al., 2010). These contribution of cholesterol to tumor movement were impaired by the reduction of cholesterol by statins (Murai et al., 2011).

Another cascade downstream of the mevalonate pathway might be important to influence cellular function or tumor survival. Farnesyl pyrophosphate is converted into geranylgeranyl pyrophosphate and both phosphates are substances of protein prenylation. Proteins acquire lipophilicity with prenylation by farnesyl transferase or geranylgeranyl transeferase and bind to membrane or hydrophobic grooves on the surface of soluble protein factors (Gelb et al., 2006). In association with tumors, the prenylation of low

molecular mass G protein has been examined, because their membrane association is induced by prenylation (Finegold et al., 1990). Lovastatin inhibits the prenylation of G proteins and alters their localization (Girgelt et al., 1994; Muller et al., 1998). Simvastatin interfered with angiogenesis via inhibition of the geranylgeranylation and membrane localization of RhoA (Park et al., 2002). RhoA activity and JNK, downstream of RhoA, were also inhibited by atorvastatin, resulting in the suppression of osteosacroma invasion (Fromigue et al., 2008). In addition, Rab protein, which was recently reported to be involved in protein transport across the secretory, was geranylgeranylated by Rab geranylgeranyl transferase, and mediated cancer invasion (Leung et al., 2006).

4.1.3 The mechanism of statins cytotoxicity; downstream of G protein

In the downstream of these low molecular mass G protein prenylations, statins induced an mTOR-dependent Ser166 phosphorylation of Mdm2, and this effect may attenuate the duration and intensity of the p53 response to DNA damage in hepatocytes (Paajarvi et al., 2005). Atorvastatins induce autophagy and autophagy-associated cell death in PC3 cells, likely through inhibition of geranylgeranylation (Parikh et al., 2010). GGTI-286, an inhibitor of geranylgeranyl transeferase, induced G0/G1 arrest and p21 resulting in tumor suppression (Vogt et al., 1997). These effects derived from p21 were related with the inhibition of histone deacetylase activity and release of promoter-associated HDAC1/2(Lin et al., 2008). Lovastatin induced U87 glioblastoma cell death in correlation with significantly increased levels of the BH3-only protein and the activation of MAPK. All of these alterations were prevented by geranylgeranyl pyrophosphate (Jiang et al., 2004). Statins have the potential to regulate gene expression related with cancer progression. MMP-9 reduction by lovastatin resulted in the suppression of invasion (Wang et al., 2000). Pitavastatin at low dose inhibits NF-kappaB activation and decreases IL-6 production induced by TNF-alpha (Wang & Kitajima, 2007). Atorvastatin inhibits inflammatory angiogenesis in mice through down-regulation of VEGF, TNF-alpha, and TGF-beta1 (Araujo et al., 2010). These reports suggest that several cancers are sensitive to statins and the inhibitors of protein prenylation.

4.1.4 The effect of statins against mesothelioma under acidic conditions

In contrast to statins' inhibitory effect against several cancers, little has been reported about the treatment of mesothelioma with statins or prenylation inhibitors. A few previous reports and our results, however, stress the possibility of mesothelioma treatment by statins. Lovastatin reduced malignant mesothelioma viability with altering the membrane association of Ras, and this cytotoxic effect was impaired by addition of mevalonate (Rubins et al., 1998). It is still unclear whether the cytotoxicity was dependent on the reduction of cholesterol or on the prenylation of proteins. In regard to the doxorubicin sensitivity of mesothelioma up-regulated by statins, the inhibition of protein prenylation was thought to potentiate the cytotoxicity of doxorubicin (Riganti et al., 2006). Both mevastatin and simvastatin increased the doxorubicin sensitivity via the increase of NO, and this effect was mimicked by GGTI-286 and Y-27632, an inhibitor of ROCK which is a downstream protein of Rho GTPase. Although, there has been no discussion about the contribution of the decreased cholesterol, the decline of cholesterol level would reduce the mitochondrial membrane potential, resulting in the release of pro-apoptotic protein. So both mechanisms are thought to contribute to the up-regulation of doxorubicin sensitivity.

Our resent results indicated that mesothelioma cells can proliferate under acidic conditions, and the pH-dependent cytotoxicity was due to the pH-dependent activity of prenylated proteins or the pH-dependent alteration of protein prenylation because manumycin A, an inhibitor of farnesyl and geranylgeranyl transferase, showed pH-dependent cytotoxicity as well as statins while no pH-dependent cytotoxicity appeared with YM-53601, an inhibitor of squalene synthase (Fukamachi et al., 2010). This importance of protein prenylation was reinforced by our result that three out of the only four inhibitors including FTI-276, an inhibitor of farnesyl transferase, are related with protein prenylation as shown in Table 2. It is not clear which protein's prenylation or activity is important in mesothelioma cells under acidic conditions. The signal transductions under acidic environments are different from those at normal tissue pH. The phosphorylation of p38 was increased at low pH (Hirata et al., 2008). The activity of the Erk/Ap-1 pathway is also activated at low pH in melanoma cells (Kato et al., 2005). These results indicate that the upstream proteins of MAPK are likely to be important at low pH. In fact, the activity of ERK in cardiac myocytes at low pH was related with Ras activation (Haworth et al., 2006). The morphological changes under acidic conditions were associated with Rho kinase (Hyvelin et al., 2004). Rab11b and its mediator Rip11 regulate V-ATPase traffic elevated at low pH in duct cells (Oehike et al., 2011). These results suggested that low molecular mass G protein might be important in tumor proliferation under acidic conditions. It remains unclear yet why statins inhibit cellular proliferation preferentially in tumor growth. Recently, a novel approach to identify geranylgeranylated protein was developed using azide binding geranylgeraniol which converted to geranylgeranyl pyrophosphate in cytosol (Chan et al., 2009). So the proteins, which play a critical role for mesothelioma proliferation at low pH, will be identified soon.

4.2 Cantharidin

4.2.1 The mechanism of cantharidin to suppress tumor growth

Cantharidin, a natural compound isolated from beetles, has the inhibitory activity of protein phosphatase (PP2A or PP1). Cantharidin was first found to be effective against various warts (Cusack et al., 2008). Cantharidine has also been traditionally used as an anti-cancer agent in China (Pang et al., 2007). The mechanism against cancer via the inhibition of protein phosphatase is poorly understood except for a few reports regarding to apoptosis and signal pathways. Cantharidin caused G2/M arrest through inhibition of CDK1 activity (Huang et al., 2011) and apoptosis via mitochondrial pathways including Bax, Bcl-2, Bcl-xl resulting in caspase activation (Kok et al., 2005). Cantharidin was recently shown to have passive cytotoxicity against tumor cells via the JAK/STAT pathway (Sagawa et al., 2008) and the involvement of NF-kB pathways in cantharidin-induced apoptosis was also reported (Li et al., 2011).

4.2.2 The problem with cantharidin and derivatives of cantharidin

Although these features of cantharidin are important for anti-tumor activity, the clinical application of cantharidin is limited due to its severe side-effects and highly toxic nature. The protein phosphatase-inhibitory toxins have been shown to induce hyper-phosphorylation of cytoskeletal proteins like keratin in isolated rat hepatocytes, and to cause disruption of the intracellular network of keratin intermediate filaments (Blankson et al., 1995). The toxins also inhibit hepatocellular processes like autophagy, endocytosis, and

protein synthesis and elicit apoptotic cell death when administered to hepatocytes in culture (Blankson et al., 2000).

Therefore, the development of more selective and effective analogs of cantharidin with less toxicity has become a challenge for cancer treatment. LB1.2, a synthesized cantharidin derivative, showed significant enhancement of cancer chemotherapy on glioblastoma and neuroblastoma cancer cells with no acute or chronic toxicity. This tumor-suppressive effect was derived from the quiescent cells cycle and caused by blocking other replication checkpoints triggered by DNA damage through the significant inhibition effect of PP2A (Rajski & Williams., 1998). Norcantharidin (NCTD) is another demethylated derivative of cantharidin possessing anti-cancer activity less toxic to normal cells, and has been used to gastric cancer (McCluskey., 2002). NCTD inhibited the activity of PP2A, and was able to promote the cell cycle from G1 to S phase with subsequent G2/M arrest (Yu et al., 2006). In addition to this phosphatase inhibitory effect, recent studies have demonstrated that cantharidin and NCTD can cause DNA damage, which may be the main contributory factor in the cytotoxicity of NCTD and cantharidin (Efferth et al., 2005). NCTD-induced caspase-dependent apoptosis was accompanied by an increase in ROS production, loss of mitochondrial membrane potential with release of cytochrome c from the mitochondria to the cytosol, and down-regulation of anti-apoptotic protein Bcl-2 (Chang et al., 2010).

4.2.3 The therapeutic capacity of cantharidin against mesothelioma

Combinational therapy with these new cantharidin derivatives may be used for treatment of mesothelioma. In combinational treatment with doxorubicin and LB1, the effectiveness of doxorubicin was greatly enhanced by the LB1 in the xenograft growth inhibition and lung metastases prevention of an aggressive sarcoma derived from transformed mesenchymal stem cells in syngeneic rats with little side toxicity (Zhang et al., 2010). Meanwhile, there have been several reports to show that cantharidin and its derivatives are relatively ineffective as an anti-cancer agent (Jiang et al., 1983). The contradiction may be related to cantharidin being less effective at alkaline pH but more active at acidic pH (Fukamachi et al., 2010)

It remains unclear why cantharidin inhibits more strongly at acidic pH and which pathways are inhibited by cantharidin under acidic environments. The protein phosphatase, which has been related with mesothelioma proliferation, is mainly PTEN (phosphatase and tensin homologue deleted from chromosome 10). The activity of PTEN was suppressed in mesothelioma (Opitz et al., 2008), and the elevated PI3K/Akt pathway, which is the target pathway of PTEN, was repeatedly confirmed and related with mesothelioma proliferation (Altomare et al., 2005). So the inhibition of PTEN by cantharidin is not likely to lead to mesothelioma death. In other cell tumors, cantharidin induced G2/M arrest through inhibition of CDK1 (Huang et al., 2011), and induced NF-kappaB activity via constitutive phosphorylation of IKK. So the sensitivity of these proteins, especially NF-kappaB, to low pH may be important because the translocation of NF-kappaB was altered dependent on extracellular pH in T cells (unpublished data). Taken together, the pharmacologic inhibition of PP2A with less-toxic cantharidin derivatives may be a useful strategy against mesothelioma as a single treatment or combined with other anti-tumor agents including doxorubicin.

4.3 The impaired effect of molecular targeted inhibitors

4.3.1 Aphidicolin

Aphidicolin is a specific inhibitor of DNA polymerase alpha and delta, resulting in DNA double-strand breaks leading to the activation of Ataxia-Telangiectasia Mutated: ATM (Ge & Blow, 2010), the inhibition of DNA replication and cell cycle arrest in G1/S phase (Dhillon et al., 2003). The impaired activity of aphidicolin at pH 6.7 shown in Table 2 and our previous result (Fukamachi et al., 2010) suggested that mesothelioma cells might have an acquired resistance to DNA double-strand breaks. Recently, an aphidicolin-resistant Chinese hamster V79 cell mutant had aphidicolin-resistant DNA polymerase that had an increased affinity for dNTP (Syljuasen et al., 2007). Furthermore, the checkpoint adaptation was observed in aphidicolin-treated Xenopus, and this system was expected to function in human cells (Herbst et al., 2003). The mechanism by which mesothelioma proliferates at pH 6.7 with aphidiconis is unclear yet. The most important point is that the ATM signal above has been thought to contribute to the exclusion of DNA mutated cells leading to tumorgenesis or tumor malignancy. Our result (Fig.2) may suggest that the extracellular acidosis influences tumor malignancy via impaired ATM signals.

4.3.2 Bisindolmaleimide

Bisindolmaleimide is a specific inhibitor of PKC (Toullec et al., 1991). PKC is a ubiquitous phospholipid-dependent serine/threonine kinase involved in major signaling events that regulate cellular growth, migration, apoptosis, and a wide variety of biological responses to stimuli (Sukumaran & Prasadarao, 2002). Several studies have indicated that the inhibition of PKC represses tumor proliferation although it depend on the PKC class (Hu et al., 2011; Toton et al., 2011). PKCbeta1 was expressed in the majority of MPM and the treatment of MPM cell lines with PKC inhibitor showed synergy when combined with cisplatin in vitro (Faoro et al., 2008). Moreover the inhibition of protein kinase C prevents asbestos-induced *c-fos* and *c-jun* proto-oncogene expression in mesothelial cells (Fung et al., 1997). Despite these results relating tumor progression with PKC, our results showed less sensitivity to Bisindolmaleimide (Table 2 & Fig.2). The suppressive acitivity of PKC at low pH was demonstrated in prostate carcinoma (Thews et al., 2006). So the impaired activity of Bisindolmaleimide may be due to the decline of PKC activity under acidic conditions.

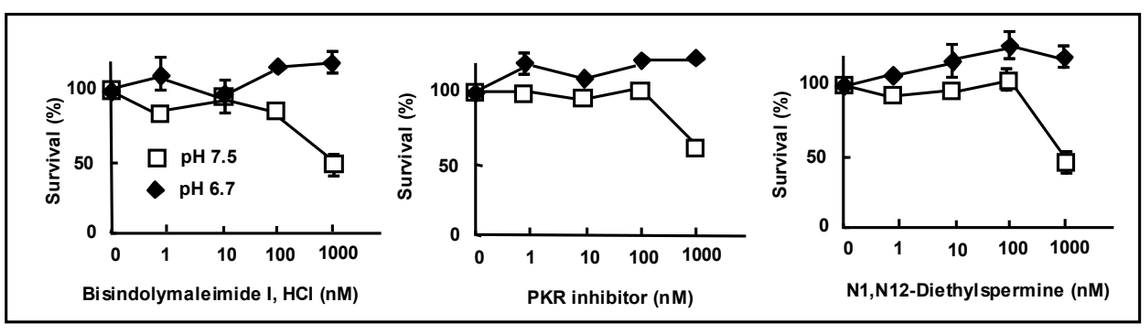

Fig. 2. pH dependency of molecular targeted inhibitors

4.3.3 PKR inhibitor

PKR, double-stranded RNA dependent protein kinase R, is activated by heme deficiency, the absence of amino acids, folded proteins accumulated in the ER, and dsRNA. The activated PKR phosphorylates eIF2α, and the phosphorylated eIF2α acts as a dominant inhibitor of the guanine exchange factor eIF2B, which prevents the recycling of eIF2 between succeeding rounds of protein synthesis and eventually leads to a global obstruction of mRNA translation initiation. This allows cells to adapt to stressful conditions by economizing on energy expended by protein synthesis (Wek et al., 2006). The adaptation process of eIF2α phoshorylation involves the selective translation of transcription factors such as activating transcription factor 4 (ATF4) (Vattem & Wek, 2004) and ATF5 (Zhou et al., 2008), which induce the expression of genes that facilitate adaptation. In cases of prolonged stress, the induction of eIF2α phosphorylation leads to cell death through the induction of apoptotic pathways (Wek et al., 2006). The results in Fig. 2 may suggest that the activity of PKR is less important in the proliferation of mesothelioma under acidic condition. Acidic conditions, in fact, enhanced the phosphorylation of eIF2, but this was confirmed at pH lower than 5.5 and not over 6.2 in a partially PKR-independent manner (Vantelon et al., 2007). So the phosphorylation of eIF2 without PKR at pH 6.7 might not cause this PKR independency at pH 6.7. Recently, there has been strong evidence to suggest that mammalian eIF2α kinases including PKR can also mediate activate glycogen synthase kinase 3 to promote the proteasomal degradation of p53 independently of eIF2α phosphorylation (Baltzis et al., 2007). So another mechanism may be critical in this reduced inhibitory effect at low pH.

4.3.4 The impaired effect of N1,N12-Diethylspermine

N1,N12-Diethylspermine lost its cytotoxic activity as shown in Table 2 and Fig. 2. N1, N12 - Diethylspermine (BESpm) caused a specific induction of spermidine/spermine N'-acetyltransferase (SSAT) activity, a cytosolic enzyme is induced in response to a variety of toxic agents, hormones and polyamine derivative (Casero et al., 1990). Polyamines are aliphatic cations present in all cells. SSAT is a rate-limiting step in polyamine catabolism, which catalyzes the transfer of the acetyl group from acetyl-CoA to the spermidine or spermine and has a predominant role in the regulation of intracellular polyamine concentrations in mammalian cells (Vujcic et al., 2000). Decreases in polyamines have been shown to promote decreased growth or apoptosis (Khan et al., 1992), depending on the cell type and the particular stimulus, suggesting a complex interaction between polyamines, cell growth, and cell death. Therefore, although polyamines are required for cell growth and differentiation, SSAT is thought to prevent overaccumulation of the higher polyamines from becoming toxic to the cell and may play a role in reducing the growth rate by decreasing intracellular polyamines. It has been shown that the regulation of SSAT by the natural polyamines and the anti-tumor polyamine analogues is through the polyamine response element (Wang et al., 1998). Furthermore, the superinduction of SSAT by polyamine analogues has been implicated in the cell type-specific cytotoxic response of several important human tumors including human lung cancer (Casero et al., 1990, 1992; Chang et al., 1992; Kim et al., 2005). Consistent with these previous reports, BESpm reduced cellular survival at pH 7.5 against mesothelioma as shown Fig. 2, probably via the inhibition of

SSAT by BESpm, leading to the desruption of polyamine regulation while no inhibitory effect was observed at pH 6.7. This result suggests that proliferation of mesothelioma cell line may be independent of the regulation of polyamine.

5. Novel anti-tumor therapy being dependent on pH

5.1 The effect of Alkylating drug

Some alkylating anti-tumor drugs including chlorambucil, carboquone and cyclophosphamide were reported to have higher cytotoxocity at low pH, probably because of their weak acid conformation or the decrease of drug resistance (Mikkelsen et al., 1985). It is difficult, however, to use them due to their causing the development of other tumors in common with alkyl agents (Rai et al., 2000).

5.2 Lowering the intracellular pH using nigericin

Low intracellular pH has been associated with enhanced cytotoxicity of anti-tumor drugs, so drugs to reduce intracellular pH have been investigated. The intracellular pH is higher than the extracellular pH, as mentioned above, so nigericin, an ionophore that acidifies the cytoplasm by exchanging cations with protons in cells placed in medium at low pH, was co-incubated with anti-tumor agents. Amiloride and 4,4'-diisothiocyanostilbene 2,2-disulfonic acid, inhibitors of the Na/H and HCO3/Cl exchangers, respectively, decreased intracellular pH in the presence of nigericin at low extracellular pH against Chinese hamster ovary and human bladder cancer MGH-U1 cells (Rotin et al., 1987).

5.3 The pH-dependent cytotoxicity of N-dodecylimidazole

N-dodecylimidazole is a compound, which acquires detergent properties under acidic conditions, and is thought to be useful for selectively killing cells under intratumor low pH environments. N-dodecylimidazole displayed pH-dependent cytotoxicity against EMT-6 and MGH-U1 cells. The cytotoxicity was enhanced 100-fold at pH 6.0 compared with pH 7.0 (Boyer et al., 1993). However, these liposomal formulations need to be optimized to achieve higher concentrations of pH-sensitive detergents within the endosome to facilitate efficient cytosolic release of liposome-entrapped contents (Chen et al., 2003). Various kinds of pH sensitive detergents have recently been developed to carry the anti-tumor drugs to the tumor nest, burst, and effuse the contents, dependent on the tumor pH. An acid-cleavable PEG lipid, 1'-(4'-cholesteryloxy-3'-butenyl)-omega-methoxy-polyethylene glycolate (CVEP), has been developed that produces stable liposomes when dispersed as a minor component (0.5-5 mol %) in 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). Cleavage of CVEP at mildly acidic pH results in dePEGylation of the latently fusogenic DOPE liposomes, thereby triggering the onset of content release (Boomer et al., 2009). Physical and chemical instabilities have limited the use of these drug carriers as pharmaceutical products. Recently, however, the preparation of freeze-dried pharmaceuticals has proven to be a successful strategy implemented to improve the stability of these formulations. Long-circulating and pH-sensitive liposomes containing Cisplatin are now being applied in vivo experiments (Giuberti et al., 2010).

5.4 The pH dependent cytotoxicity of tirapazamine

Low pH can substantially potentiate the cytotoxic effect of the bioreductive drug tirapazamine in HT-29 human tumor cells (Skarsgard et al., 1993). Tirapazamine is the lead member of a class of bioreductive drugs and requires metabolic activation to give a cytotoxic free radical species via a variety of cellular reductases, including NADPH cytochrome c reductase (Chinje et al., 2003). Combination therapy with tirapazamine and cisplatin has shown increased treatment efficacy compared with cisplatin alone in malignant melanoma and non-small-cell lung cancer, and also may be of benefit when combined with both radiotherapy and cisplatin in head and neck cancer (Williams et al., 2001).

6. Conclusion

As discussed in this review, there are no classical anti-tumor drugs which show high cytotoxicity against mesothelioma at pH 6.7. So it might be much important to prescribe both drugs whose cytotoxicity is enhanced at low pH and drugs which induce cell death at normal tissue pH for mesothelioma treatment. Although classical anti-tumor medicines have potent tumor suppressive effects, they also have severe toxicities for normal tissues. So the therapeutic regimes with lesser amounts of classical anti-tumor agents are urgently required. To this end, drugs which show higher cytotoxicity at low pH, including statins and cantharidins would be conventional candidates for co-treatment of mesothelioma. In fact, the combination treatment of solid tumors with statins enhanced anti-tumor drugs in vivo (Agarwal et al., 1999; Gao et al., 2010; O'Brien et al. 2003; Zhao et al., 2010). So the development of new compounds with anti-tumor activity preferentially at low pH would be useful for chemotherapy of mesothelioma.

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8. References

Agarwal, B., Bhendwal, S., Halmos, B., Moss, F., Ramey, G. & Holt, R. (1999) Lovastatin augments apoptosis induced by chemotherapeutic agents in colon cancer cells. *Clin Cancer Res.* Vol.5, No.8, pp.2223, ISSN 1078-2432

Alli, E., Sharma, B., Hartman, R., Lin, S.,McPherson, L. & Ford, M. (2011) Enhanced sensitivity to cisplatin and gemcitabine in Brca1-deficient murine mammary epithelial cells. *BMC Pharmacol.* Vol.11, pp.7, ISSN 1471-2210

Altomare, A., You, H., Xiao, H., Ramos-Nino, E., Skele, L., Rienzo, A., Jhanwar, C., Mossman, T., Kane, B. & Testa, R. (2005) Human and mouse mesotheliomas exhibit elevated AKT/PKB activity, which can be targeted pharmacologically to inhibit tumor cell growth. *Oncogene*. Vol.24, No.40, pp.6080, ISSN 0950-9232

Araújo, A., Rocha, A., Mendes, B. & Andrade, P. (2010) Atorvastatin inhibits inflammatory angiogenesis in mice through down regulation of VEGF, TNF-alpha and TGF-beta1. *Biomed Pharmacother.* Vol.64, No.1, pp.29, ISSN 0753-3322

Ardizzoni, A., Rosso, R., Salvati, F., Fusco, V., Cinquegrana, A., De Palma, M., Serrano, J., Pennucci, MC., Soresi,, E. & Crippa, M., et al. (1991) Activity of doxorubicin and cisplatin combination chemotherapy in patients with diffuse malignant pleural mesothelioma. An Italian Lung Cancer Task Force (FONICAP) Phase II study. *Cancer.* Vol.67, No.12, pp.2984, ISSN 1097-0142

Ballantyne, CM., Corsini, A., Davidson, MH., Holdaas, H., Jacobson, TA., Leitersdorf, E., März, W., Reckless, JP. & Stein, EA. (2003) Risk for myopathy with statin therapy in high-risk patients. *Arch Intern Med.* Vol.163, No.5, pp.553, ISSN 0003-9926

Baltzis, D., Pluquet, O., Papadakis, I., Kazemi, S., Qu, K. & Koromilas, E. (2007) The eIF2α kinases PERK and PKR activate glycogen synthase kinase 3 to promote the proteasomal degradation of p53. *J Biol Chem*. Vol.282, No.43, pp.31675, ISSN 0021-9528

Blankson, H., Holen, I., & Seglen, O. *(*1995*)* Disruption of the cytokeratin cytoskeleton and inhibition of hepatocytic autophagy by okadaic acid. *Exp. Cell Res.* Vol.218, pp522–530. ISSN 0014-4827

Blankson, H., Grotterød, M. & Seglen, O. (2000) Prevention of toxin-induced cytoskeletal disruption and apoptotic liver cell death by the grapefruit flavonoid, naringin. *Cell Death Differ.* Vol.7, pp. 739–746. ISSN 1350-9047

Boyer, J., Horn, I., Firestone, A., Steele-Norwood, D. & Tannock, F. (1993) pH dependent cytotoxicity of N-dodecylimidazole: a compound that acquires detergent properties under acidic conditions. *Br J Cancer.* Vol.67, No.1, pp.81, ISSN 0007-0920

Boomer, JA., Qualls, MM., Inerowicz, HD., Haynes, RH., Patri, VS., Kim, JM. & Thompson, DH. (2009) Cytoplasmic delivery of liposomal contents mediated by an acid-labile cholesterol-vinyl ether-PEG conjugate. *Bioconjug Chem*.. Vol.20, No.1, pp47, ISSN 1043-1802

Brugts, J., Yetgin, T., Hoeks, E., Gotto, M., Shepherd, J., Westendorp, G., de Craen, J., Knopp, H., Nakamura, H., Ridker, P., Domburg, R. & Deckers, JW. (2009) The benefits of statins in people without established cardiovascular disease but with cardiovascular risk factors: meta-analysis of randomised controlled trials. *BMJ.* Vol.338, pp.2376, ISSN 1759-2151

Casero, A., Celano, P., Ervin, J., Wiest, L. & Pegg, E. (1990) High specific induction of spermidine/spermine N1-acetyltransferase in a human large cell lung carcinoma. *Biochem J.* Vol.270, No.3, pp.615, ISSN 0264-6021

Casero, A., Mank, R., Xiao, L., Smith, J., Bergeron, J., & Celano, P. (1992) Steady-state messenger RNA and activity correlates with sensitivity to N1,N12-bis(ethyl)spermine in human cell lines representing the major forms of lung cancer. *Cancer Res*. Vol.52, No.5359, pp.5363, ISSN 0008-5472

Chan, N., Hart, C., Guo, L., Nyberg, T., Davies, S., Fong, G., Young, G., Agnew, J. & Tamanoi, F. (2009) A novel approach to tag and identify geranylgeranylated proteins. *Electrophoresis.* Vol.30, No.20, pp.3598, ISSN 0173-0835

Chang, K., Bergeron, J., Porter, W., Vinson, R., Liang, Y., & Libby, R. (1992) Regulatory and antiproliferative effects of N-alkylated polyamine analogues in human and hamster pancreatic adenocarcinoma cell lines. *Cancer Chemother. Pharmacol.* Vol.30, No.183, pp.188, ISSN 0344-5704

Chang, C., Zhu, Q., Mei, J., Liu, Q. & Luo, J. (2010) Involvement of mitochondrial pathway in NCTD-induced cytotoxicity in human hepG2 cells. *J Exp Clin Cancer Res.* Vol.29, pp.145, ISSN 0392-9078

Chen, J., Asokan, A. & Cho, J. (2003) Cytosolic delivery of macromolecules: I. Synthesis and characterization of pH-sensitive acyloxyalkylimidazoles. *Biochim Biophys Acta.* Vol.1611, No.1-2, pp.140, ISSN 0006-3002

Chinje, C., Cowen, L., Feng, J., Sharma, P., Wind, S., Harris, L. & Stratford, J. (2003) Non-nuclear localized human NOSII enhances the bioactivation and toxicity of tirapazamine (SR4233) in vitro. *Mol Pharmacol.* Vol.63, No.6, pp.1248, ISSN 0026-895X

Cho, SJ., Kim, JS., Kim, JM., Lee, JY., Jung, HC. & Song, IS. (2008) Simvastatin induces apoptosis in human colon cancer cells and in tumor xenografts, and attenuates colitis-associated colon cancer in mice. *Int J Cancer.* Vol.123, No.4, pp.951, ISSN 0020-7136

Cusack, C., Fitzgerald, D., Clayton, TM. & Irvine, AD. (2008) Successful treatment of florid cutaneous warts with intravenous cidofovir in an 11-year-old girl. *Pediatr Dermatol.* Vol.25, No.3, pp.387, ISSN 0736-8046

Dhillon, S., Husain, A. & Ray, N. (2003) Expression of aphidicolin-induced fragile sites and their relationship between genetic susceptibility in breast cancer, ovarian cancer, and non-small-cell lung cancer patients. *Teratog Carcinog Mutagen.* Vol.1, pp.35, ISSN 0270-3211

Edlow, D. W., & Sheldon, W. H. (1971) The pH of inflammatory exudates. *Proc. Soc. Exp. Biol. Med.* Vol. 137, pp. 1328, ISSN 0037-9727

Efferth, T., Rauh, R., Kahl, S., Tomicic, M., Böchzelt, H., Tome, E., Briehl, M., Bauer, R. & Kaina, B. (2005) Molecular modes of action of cantharidin in tumor cells. *Biochem Pharmacol.* Vol.69, No.5, pp.811, ISSN 0006-2952

Elbling, L., Berger, W., Weiss, RM., Printz, D., Fritsch, G. & Micksche, M. (1998) A novel bioassay for P-glycoprotein functionality using cytochalasin D. *Cytometry.* Vol.31, No.3, pp.187, ISSN 1552-4922

Faoro, L., Loganathan, S., Westerhoff, M., Modi, R., Husain, N., Tretiakova, M., Seiwert, T., Kindler, L., Vokes, E. & Salgia, R. (2008) Protein kinase C beta in malignant pleural mesothelioma. *Anticancer Drugs.* Vol.19, No.9, pp.841, ISSN 0959-4973

Fennell, DA., Steele, JP., Shamash, J., Evans, MT., Wells, P., Sheaff, MT., Rudd, RM. & Stebbing, J. (2007) Efficacy and safety of first- or second-line irinotecan, cisplatin, and mitomycin in mesothelioma. *Cancer.* Vol.109, No.1, pp.93, ISSN 1097-0142

Finegold, A., Schafer, R., Rine, J., Whiteway, M. & Tamanoi, F. (1990) Common modifications of trimeric G proteins and ras protein: involvement of polyisoprenylation. *Science.* Vol.249, No.4965, pp.165, ISSN 0036-8075

Fukamachi, T., Saito, H., Kakegawa, T. & Kobayashi, H. (2002) Different proteins are phosphorylated under acidic environments in Jurkat cells. *Immunol Lett.* Vol.82, No.1-2, pp.155, ISSN 0165-2478

Fukamachi, T., Chiba, Y., Wang, X., Saito, H., Tagawa, M. & Kobayashi, H. (2010) Tumor specific low pH environments enhance the cytotoxicity of lovastatin and cantharidin. *Cancer Lett.* Vol.297, No.2, pp182, ISSN 0304-3835

Fung, H., Quinlan, R., Janssen, M., Timblin, R., Marsh, P., Heintz, H., Taatjes, J., Vacek, P., Jaken, S. & Mossman, T. (1997) Inhibition of protein kinase C prevents asbestos-induced c-fos and c-jun proto-oncogene expression in mesothelial cells. *Cancer Res.* Vol.57, No.15, pp.3101, ISSN 0008-5472

Fromigué, O., Hamidouche, Z. & Marie, J. (2008) Blockade of the RhoA-JNK-c-Jun-MMP2 cascade by atorvastatin reduces osteosarcoma cell invasion. *J Biol Chem.* Vol.283, No.45, pp.30549, ISSN 0021-9258

Gaertner, LS., Murray, CL. & Morris, CE. (1998) Transepithelial transport of nicotine and vinblastine in isolated malpighian tubules of the tobacco hornworm (Manduca sexta) suggests a P-glycoprotein-like mechanism. *Exp Biol.* Vol.201, No.18, pp.2637, ISSN 0176-8638

Gao, J., Jia, D., Li, S., Wang, W., Xu, L., Ma, L., Ge, S., Yu, H., Ren, H., Liu, B. & Zhang, H. (2010) Combined inhibitory effects of celecoxib and fluvastatin on the growth of human hepatocellular carcinoma xenografts in nude mice. *J Int Med Res.* Vol.38, No.4, pp.1413, ISSN 0300-0605

Garwood, R., Kumar, S., Baehner, L., Moore, H., Au, A., Hylton, N., Flowers, I., Garber, J., Lesnikoski, A., Hwang, S., Olopade, O., Port, R., Campbell, M. & Esserman, J. (2010) Fluvastatin reduces proliferation and increases apoptosis in women with high grade breast cancer. *Breast Cancer Res Treat.* Vol.119, No.1, pp.137, ISSN 0167-6806

Ge, Q. & Blow, J. (2010) Chk1 inhibits replication factory activation but allows dormant origin firing in existing factories. *J Cell Biol.* Vol.191, No.7, pp.1285, ISSN 0021-9525

Gelb, H., Brunsveld, L., Hrycyna, A., Michaelis, S., Tamanoi, F., Van, C. & Waldmann, H. (2006) Therapeutic intervention based on protein prenylation and associated modifications. *Nat Chem Biol.* Vol.2, No.10, pp.518, ISSN 1552-4469

Gerson, J., MacDonald, S., Alberts, W., Kornbrust, J., Majka, A., Stubbs, J., Bokelman, L. (1989) Animal safety and toxicology of simvastatin and related hydroxy-methylglutaryl-coenzyme A reductase inhibitors. *Am J Med.* Vol.87, No.4A, pp.28S, ISSN 0002-9343

Gerweck, LE., & Seetharaman, K. (1996) Cellular pH gradient in tumor versus normal tissue: potential exploitation for the treatment of cancer. *Cancer Res.* Vol.56. No.6, pp.1194, ISSN 0008-5472

Girgert, R., Marini, P., Janessa, A., Bruchelt, G., Treuner, J. & Schweizer, P. (1994) Inhibition of the membrane localization of p21 ras proteins by lovastatin in tumor cells possessing a mutated N-ras gene. *Oncology.* Vol.51, No.4, pp.320, ISSN 2156-6976

Giuberti, C., Reis, C., Rocha, G., Leite, A., Lacerda, G., Ramaldes, A. & Oliveira, C. (2011) Study of the pilot production process of long-circulating and pH-sensitive liposomes containing cisplatin. *J Liposome Res.* Vol.21, No.1, pp.60, ISSN 0898-2104

Hahn, GM. & Shiu, EC. (1983) Effect of pH and elevated temperatures on the cytotoxicity of some chemotherapeutic agents on Chinese hamster cells in vitro. *Cancer Res.* Vol.43, No.1, pp.5789, ISSN 0008-5472

Harvey, VJ., Slevin, ML., Ponder, BA., Blackshaw, AJ. & Wrigley, PF., (1984) Chemotherapy of diffuse malignant mesothelioma. Phase II trials of single-agent 5-fluorouracil and adriamycin. *Cancer.* Vol.54, No.6, pp.961, ISSN 1097-0142

Haworth, S., Dashnyam, S. & Avkiran, M. (2006) Ras triggers acidosis-induced activation of the extracellular-signal-regulated kinase pathway in cardiac myocytes. *Biochem J.* Vol.399, No.3, pp.493, ISSN 0264-6021

Hedges, KL., Morré, DM., Wu, LY. & Morre, DJ. (2003). Adriamycin tolerance in human mesothelioma lines and cell surface NADH oxidase. *Life Sci.* Vol.73, No.9, pp.1189, ISSN 0024-3205

Herbst, S. & Kies, S. (2003) Gefitinib: current and future status in cancer therapy. *Clin. Adv. Hematol. Oncol,* Vol.1, pp.466, ISSN 1543-0790

Hirata, S., Fukamachi, T., Sakano, H., Tarora, A., Saito, H. & Kobayashi, H. (2008) Extracellular acidic environments induce phosphorylation of ZAP-70 in Jurkat T cells. *Immunol Lett*. Vol.115, No.2, pp.105, ISSN 0165-2478

Hoque, A., Chen, H., Xu & XC. (2008) Statin induces apoptosis and cell growth arrest in prostate cancer cells. *Cancer Epidemiol Biomarkers Prev.* Vol.17, No.1, pp.88, ISSN 1055-9965

Hu, T., Wu, R., Cheng, C., Wang, S., Wang, T., Lee, C., Wang, J., Pan, M., Chang, Y. & Wu, S. (2011) Reactive oxygen species-mediated PKC and integrin signaling promotes tumor progression of human hepatoma HepG2. *Clin Exp Metastasis.* [Epub ahead of print] ISSN 0262-0898

Huang, WW., Ko, SW. & Tsai, HY., et al. (2011) Cantharidin induces G2/M phase arrest and apoptosis in human colorectal cancer colo 205 cells through inhibition of CDK1 activity and caspase-dependent signaling pathways. *Int J Oncol.* Vol.38, No.4, pp.1067, ISSN 0020-7136

Hyvelin, M., O'Connor, C. & McLoughlin, P. (2004) Effect of changes in pH on wall tension in isolated rat pulmonary artery: role of the RhoA/Rho-kinase pathway. *Am J Physiol Lung Cell Mol Physiol.* Vol.287, No.4, pp.L673, ISSN 1040-0605

Isobe, H., Wellham, L., Sauerteig, A., Sridhar, KS., Ramachandran, C. & Krishan, A. (1994) Doxorubicin retention and chemoresistance in human mesothelioma cell lines. *Int J Cancer.* Vol.57, No.4, pp.581, ISSN 0020-7136

Jähde, E., Glüsenkamp, KH. & Rajewsky, MF. (1990) Protection of cultured malignant cells from mitoxantrone cytotoxicity by low extracellular pH: a possible mechanism for chemoresistance in vivo. *Eur J Cancer.* Vol.26, No.26, pp.101, ISSN 0959-8049

Jähde, E., Volk, T., Atema, A., Smets, LA., Glüsenkamp, KH. & Rajewsky, MF. (1992) pH in human tumor xenografts and transplanted rat tumors: effect of insulin, inorganic phosphate, and m-iodobenzylguanidine. *Cancer Res.* Vol. 52, No. 22, pp.6209, ISSN 0008-5472

Jiang, L., Salmon, E. & Liu, M. (1983) Activity of camptothecin, harringtonin, cantharidin and curcumae in the human tumor stem cell assay. *Eur J Cancer Clin Oncol* Vol.19, No.2, pp.263, ISSN 0277-5379

Jiang, Z., Zheng, X., Lytle, A., Higashikubo, R. & Rich, M. (2004) Lovastatin-induced up-regulation of the BH3-only protein, Bim, and cell death in glioblastoma cells. *J Neurochem.* Vol.89, No.1, pp.168, ISSN 0022-3042

Jones, KD., Couldwell, WT., Hinton, DR., Su, Y., He, S., Anker, L. & Law, RE. (1994) Lovastatin induces growth inhibition and apoptosis in human malignant glioma cells. *Biochem Biophys Res Commun.* Vol.205, No.3, pp.1681, ISSN 0006-291X

Kahlos, K., Anttila, S., Asikainen, T., Kinnula, K., Raivio, KO., Mattson, K., Linnainmaa, K. & Kinnula, VL.(1998) Manganese superoxide dismutase in healthy human pleural mesothelium and in malignant pleural mesothelioma. *Am J Respir Cell Mol Biol.*Vol.18, No.4, pp.570, ISSN 1044-1549

Kato, Y., Lambert, CA., Colige, AC., Mineur, P., Noël, A., Frankenne, F., Foidart, JM., Baba, M., Hata, R., Miyazaki, K. & Tsukuda, M. (2005) Acidic extracellular pH induces matrix metalloproteinase-9 expression in mouse metastatic melanoma cells through the phospholipase D-mitogen-activated protein kinase signaling. *J Biol Chem.* Vol.280, No.12, pp10938, ISSN 0021-9258

Kawata, S., Kakimoto, H., Ishiguro, H., Yamasaki, E., Inui, Y. & Matsuzawa, Y. (1992) Effect of pravastatin, a potent 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor, on survival of AH130 hepatoma-bearing rats. *Jpn J Cancer Res.* Vol.83, No.11, pp.1120, ISSN 0910-5050

Khan, U., Mei, H. & Wilson, T. (1992) A proposed function for spermine and spermidine: protection of replicating DNA against damage by singlet oxygen. *Proc. Natl. Acad. Sci. U.S.A.* Vol.89, No.23, pp.11426, ISSN 0027-8424

Kim, K., Ryu, H., Park, W., Kim, S. & Chun, S. (2005) Induction of a SSAT isoform in response to hypoxia or iron deficiency and its protective effects on cell death. *Biochem.Biophys. Res. Commun*. Vol.331, No.7, pp85, ISSN 0006-291X

Kok, H., Cheng, J. & Hong, Y, et al. (2005) Norcantharidin-induced apoptosis in oral cancer cells is associated with an increase of proapoptotic to antiapoptotic protein ratio. *Cancer Lett.* Vol.217, No.1, pp.43, ISSN 0304-3835

Kusama, T., Mukai, M., Iwasaki, T., Tatsuta, M., Matsumoto, Y., Akedo, H., Inoue, M. & Nakamura, H. (2002) 3-hydroxy-3-methylglutaryl-coenzyme a reductase inhibitors reduce human pancreatic cancer cell invasion and metastasis. *Gastroenterology.* Vol.122, No.2, pp.308, ISSN 0016-5085

Lagadic-Gossmann, D., Huc, L. & Lecureur,.V. (2004) Alterations of intracellular pH homeostasis in apoptosis: origins and roles. *Cell Death Differ.* Vol.11, No.9, pp.953, ISSN 1350-9047

Lao, Q., Kuge, O., Fukamachi, T., Kakegawa, T., Saito, H., Nishijima, M. & Kobayashi, H. (2005) An IkappaB-beta COOH terminal region protein is essential for the proliferation of CHO cells under acidic stress. *J Cell Physiol*. Vol.203, NO.1, pp.186, ISSN 0021-9541

Lao, Q., Fukamachi, T., Saito, H., Kuge, O., Nishijima, M. & Kobayashi, H. (2006) Requirement of an IkappaB-beta COOH terminal region protein for acidic-adaptation in CHO cells. *J Cell Physiol*. Vol.207 NO.1, pp.238, ISSN 0021-9541

Laurencot, CM. & Kennedy, KA. (1995) Influence of pH on the cytotoxicity of cisplatin in EMT6 mouse mammary tumor cells. *Oncol Res.* Vol. 7-8, No., pp.371, ISSN 0965-0407

Lee, J., Jung, H., Park, S., Ahn, B., Shin, J., Im, A., Oh do, Y., Shin, B., Kim, W., Lee, N., Byun, H., Hong, S., Park, O., Park, H., Lim, Y. & Kang, K. (2009) Simvastatin plus irinotecan, 5-fluorouracil, and leucovorin (FOLFIRI) as first-line chemotherapy in metastatic colorectal patients: a multicenter phase II study. *Cancer Chemother Pharmacol.* Vol.64, No.4, pp.657, ISSN 0344-5704

Leung, F., Baron, R. & Seabra, C. (2006) Thematic review series: lipid posttranslational modifications. geranylgeranylation of Rab GTPases. *J Lipid Res.* Vol.47, No.3, pp.467, ISSN 0022-2275

Li, W., Chen, Z. & Zong, Y. (2011) PP2A inhibitors induce apoptosis in pancreatic cancer cell line PANC-1 through persistent phosphorylation of IKK and sustained activation of the NF-B pathway. *Cancer Lett.* Vol.304, No.2, pp.117, ISSN 0304-3835

Lin, C., Lin, H., Chou, W., Chang, F., Yeh, H. & Chen, C. (2008) Statins increase p21 through inhibition of histone deacetylase activity and release of promoter-associated HDAC1/2. *Cancer Res.* Vol.68, No.7, pp.2375, ISSN 0008-5472

Lu, J., Zhuang, Z., Song, DK., Mehta,GU., Ikejiri,B., Mushlin,H., Park,DM. &Lonser, RR. (2010) The effect of a PP2A inhibitor on the nuclear receptor corepressor pathway in glioma. *J Neurosurg.* Vol.113. No.2. pp225, ISSN 0022-3085

Maksimova, E., Yie, TA. & Rom, WN. (2008) In vitro mechanisms of lovastatin on lung cancer cell lines as a potential chemopreventive agent. *Lung.* Vol.186, No.1, pp.45, ISSN 0341-2040

McCluskey, A., Sim, T. & Sakoff, A. (2002) Serine-threonine protein phosphatase inhibitors: development of potential therapeutic strategies. *J Med Chem.* Vol.45, No.6, pp.1151, ISSN 0022-2623

Mekhail, K., Gunaratnam, L., Bonicalzi, ME. & Lee, S. (2004) HIF activation by pH-dependent nucleolar sequestration of VHL. *Nat Cell Biol.* Vol.6, No.7, pp.642, ISSN 1097-6256

Montero, J., Morales, A., Llacuna, L., Lluis, M., Terrones, O., Basañez, G., Antonsson, B., Prieto, J., García-Ruiz, C., Colell, A. & Fernández-Checa, C. (2008) Mitochondrial cholesterol contributes to chemotherapy resistance in hepatocellular carcinoma. *Cancer Res.* Vol.68, No.13, pp.5246, ISSN 0008-5472

Müller, C., Bockhorn, AG., Klusmeier, S., Kiehl, M., Roeder, C., Kalthoff, H. & Koch, OM. (1998) Lovastatin inhibits proliferation of pancreatic cancer cell lines with mutant as well as with wild-type K-ras oncogene but has different effects on protein phosphorylation and induction of apoptosis. *Int J Oncol.* Vol.12, No.3, pp.717, ISSN 1019-6439

Murai, T., Maruyama, Y., Mio, K., Nishiyama, H., Suga, M. & Sato, C. (2011) Low cholesterol triggers membrane microdomain-dependent CD44 shedding and suppresses tumor cell migration. *J Biol Chem.* Vol.286, No.3, pp.1999, ISSN 0021-9258

Newman, A., Clutterbuck, RD., Powles, RL. & Millar, JL. (1994) Selective inhibition of primary acute myeloid leukaemia cell growth by lovastatin. *Leukemia.* Vol.8, No.2, pp.274, ISSN 0887-6924

Nissen, E. & Tanneberger, S. (1981) Influence of pH and serum on the effectivity of antineoplastic agents in vitro. Arch Geschwulstforsch. Vol. 51, No. 1, pp152, ISSN 0003-911x

O'Brien, G., Meinhardt, P. & Bond, E., et al. (2003) Effects of imatinib mesylate (STI571, Glivec) on the pharmacokinetics of simvastatin, a cytochrome p450 3A4 substrate,

in patients with chronic myeloidleukaemia. *Br J Cancer.* Vol89., No.10, pp.1855, ISSN 0007-0920

Oehlke, O., Martin, W., Osterberg, N., Roussa, E. (2011) Rab11b and its effector Rip11 regulate the acidosis-induced traffic of V-ATPase in salivary ducts. *J Cell Physiol.* Vol.226, No.3, pp.638, ISSN 0021-9541

Opitz, I., Soltermann, A., Abaecherli, M., Hinterberger, M., Probst-Hensch, N., Stahel, R., Moch, H. & Weder, W. (2008) PTEN expression is a strong predictor of survival in mesothelioma patients. *Eur J Cardiothorac Surg.* Vol.33, No.3, pp.502, ISSN 1010-7940

Owen, CS., Pooler, PM., Wahl, ML., Coss, RA. & Leeper, DB. (1997) Altered proton extrusion in cells adapted to growth at low extracellular pH. *J Cell Physiol.* Vol.173, No.3, pp.397, ISSN 0021-9541

Pääjärvi, G., Roudier, E., Crisby, M., Högberg, J. & Stenius, U. (2005) HMG-CoA reductase inhibitors, statins, induce phosphorylation of Mdm2 and attenuate the p53 response to DNA damage. *FASEB J.* Vol.19, No.3, pp.476, ISSN 0892-6638

Pang, K., Yu, W., Au-Yeung, C. & Ho, P. (2007) DNA damage induced by novel Demethylcantharidin – integrated platinum anticancer complexes. *Biochem Biophys Res Commun.* Vol.363, No.1, pp.235, ISSN 0006-291X

Parikh, A., Childress, C., Deitrick, K., Lin, Q., Rukstalis, D. & Yang, W. (2010) Statin-induced autophagy by inhibition of geranylgeranyl biosynthesis in prostate cancer PC3 cells. *Prostate.* Vol.70, No.9, pp.971, ISSN 0270-4137

Park, J., Kong, D., Iruela-Arispe, L., Begley, U., Tang, D. & Galper, B. (2002) 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors interfere with angiogenesis by inhibiting the geranylgeranylation of RhoA. *Circ Res.* Vol.91, No.2, pp.143, ISSN 0009-7330

Pope, BJ., Zierler-Gould, KM., Kühne, R., Weeds, AG. & Ball, LJ. (2004) Solution structure of human cofilin: actin binding, pH sensitivity, and relationship to actin-depolymerizing fact0or. *J Biol Chem.* Vol.279, No.6, pp.4840, ISSN 0021-9528

Raghunand, N., He, X., van Sluis, R., Mahoney, B., Baggett, B., Taylor, CW., Paine-Murrieta, G., Roe, D., Bhujwalla, ZM. & Gillies RJ. (1999) Enhancement of chemotherapy by manipulation of tumour pH. *Br J Cancer* .Vol.80, No.7, pp.1005, ISSN 0007-0920

Rai, R., Peterson, L., Appelbaum, R., Kolitz, J., Elias, L., Shepherd, L., Hines, J., Threatte, A., Larson, A., Cheson, D. & Schiffer, A. (2000) Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. *N Engl J Med.* Vol.343, No.24, pp.1750, ISSN 0028-4793

Rajski, R. & Williams, M. (1998) DNA Cross-Linking Agents as Antitumor Drugs. *Chem Rev.* Vol.98, No.8, pp.2723, ISSN 0009-2665

Riganti, C., Orecchia, S., Pescarmona, G., Betta, G., Ghigo, D. & Bosia, A. (2006) Statins revert doxorubicin resistance via nitric oxide in malignant mesothelioma. *int J Cancer*. Vol.119, No.1, pp.17, ISSN 0020-7136

Riganti, C., Doublier, S., Aldieri, E., Orecchia, S., Betta, PG., Gazzano, E., Ghigo, D. & Bosia, A. (2008) Asbestos induces doxorubicin resistance in MM98 mesothelioma cells via HIF-1alpha. *Eur Respir J.* Vol.32, No.2, pp.443, ISSN 0903-1936

Rockwells, S. (1986) Effect of some proliferative and environmental factors on the toxicity of mitomycin C to tumor cells in vitro. *Int J Cancer.* Vol.38, No.2, pp.229, ISSN 0020-7136

Rofstad, EK., Mathiesen, B., Kindem, K. & Galappathi, K. (2006) Acidic extracellular pH promotes experimental metastasis of human melanoma cells in athymic nude mice. *Cancer Res*. Vol.66, No.13, pp.6699, ISSN 0008-5472

Rogalska, A., Koceva-Chyła, A. & Jóźwiak, Z. (2008) Aclarubicin-induced ROS generation and collapse of mitochondrial membrane potential in human cancer cell lines. *Chem Biol Interact.* Vol.176, No.1, pp.58, ISSN 0009-2797

Rotin, D., Wan, P., Grinstein, S. & Tannock, I. (1987) Cytotoxicity of compounds that interfere with the regulation of intracellular pH: a potential new class of anticancer drugs. *Cancer Res.* Vol.47, No.6, pp.1497, ISSN 0008-5472

Rubins, B., Greatens, T., Kratzke, A., Tan, T., Polunovsky, A. & Bitterman, P. (1998) Lovastatin induces apoptosis in malignant mesothelioma cells. *Am J Respir Crit Care Med.* Vol.157, No.1, pp.1616, ISSN 1073-449X

Rueth, NM., Murray, SE., Huddleston, SJ., Abbott, AM., Greeno, EW., Kirstein, MN. & Tuttle, TM. (2011) Severe electrolyte disturbances after hyperthermic intraperitoneal chemotherapy: oxaliplatin versus mitomycin C. *Ann Surg Oncol.* Vol.18, No.1, pp.174, ISSN 1068-9265

Ruiz-Gómez, J., Souviron, A., Martínez-Morillo, M. & Gil, L. (2000) P-glycoprotein, glutathione and glutathione S-transferase increase in a colon carcinoma cell line by colchicine. *J Physiol Biochem.* Vol.56, No.4, pp.307, ISSN 1138-7548

Sagawa, M., Nakazato, T., Uchida, H., Ikeda, Y. & Kizaki, M. (2008) Cantharidin induces apoptosis of human multiple myeloma cells via inhibition of the JAK/STAT pathway. *Cancer Sci.* Vol.99, No.9, pp.1820, ISSN 1347-9032

Sauvant, C., Nowak, M., Wirth, C., Schneider, B., Riemann, A., Gekle, M. & Thews, O. (2008) Acidosis induces multi-drug resistance in rat prostate cancer cells (AT1) in vitro and in vivo by increasing the activity of the p-glycoprotein via activation of p38, *Int. J. Cancer.* Vol.123, pp.2532, ISSN 0020-7136

SCADs inhibitor kit 1, Available from: < http://scads.jfcr.or.jp/file/kit1ver-2.3.xls>

SCADs inhibitor kit 2, Available from: < http://scads.jfcr.or.jp/file/kit2.xls>

SCADs inhibitor kit 3, Available from: < http://scads.jfcr.or.jp/file/kit3-1.2.xls>

Sekine, Y., Demosky, J., Stonik, A, Furuya, Y., Koike, H., Suzuki, K. & Remaley, T. (2010) High-density lipoprotein induces proliferation and migration of human prostate androgen-independent cancer cells by an ABCA1-dependent mechanism. *Mol Cancer Res.* Vol.8, No.9, pp.1284, ISSN 1541-7786

Schliwa, M. (1982) Action of cytochalasin D on cytoskeletal networks. *J Cell Biol.* Vol.92, No.1, pp.79, ISSN 0021-9525

Shannon, HE., Martin, WR. & Silcox, D. (1978) Lack of antiemetic effects of delta9-tetrahydrocannabinol in apomorphine-induced emesis in the dog. *Life Sci.* Vol.23, No.1, pp.49, ISSN 0024-3205

Simmen, H. P. (1993) J. Blaser. Analysis of pH and pO_2 in abscesses, peritoneal fluid, and drainage fluid in the presence or absence of bacterial infection during and after abdominal surgery. *Am. J. Surg.* Vol.166, pp.24, ISSN 0147-5185

Skarsgard, D., Skwarchuk, W., Vinczan, A. & Chaplin, J. (1993) The effect of pH on the aerobic and hypoxic cytotoxicity of SR4233 in HT-29 cells. *Br J Cancer.* Vol.,68 No.4, pp.681, ISSN 0007-0920

Steele, JP., O'Doherty, CA., Shamash, J., Evans, MT., Gower, NH., Tischkowitz, MD. & Rudd, RM. (2001) Phase II trial of liposomal daunorubicin in malignant pleural mesothelioma. *Ann Oncol.* Vol.12, No.4, pp.497, ISSN 0923-7534

Stewart, DJ., Gertler, SZ., Tomiak, A., Shamji, F., Goel, R. & Evans WK. (1994) High dose doxorubicin plus cisplatin in the treatment of unresectable mesotheliomas: report of four cases. *Lung Cancer*. Vol.11, No.3-4, pp.251, ISSN 0169-5002

Sukumaran, K. & Prasadarao, V. (2002) Regulation of protein kinase C in Escherichia coli K1 invasion of human brain microvascular endothelial cells. *J Biol Chem.* Vol.277, No.14, pp.12253, ISSN 0021-9258

Syljuåsen, R.G. (2007) Checkpoint adaptation in human cells. *Oncogene.* Vol.26, pp.5833, ISSN 0950-9232

Thews, O., Gassner, B., Kelleher, DK., Schwerdt, G. & Gekle, M. (2006) Impact of extracellular acidity on the activity of P-glycoprotein and the cytotoxicity of chemotherapeutic drugs. *Neoplasia*. Vol.8, No.2, pp.143, ISSN 1552-8002

Tobert, JA. (2003) Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. *Nat Rev Drug Discov.* Vol.2, No.7, pp.517, ISSN 1474-1776

Totoń, E., Ignatowicz, E., Skrzeczkowska, K. & Rybczyńska, M. (2011) Protein kinase Cε as a cancer marker and target for anticancer therapy. *Pharmacol Rep.* Vol.63, No.1, pp.19, ISSN 1734-1140

Toullec, D., Pianetti, P., Coste, H., Bellevergue, P., Grand-Perret, T., Ajakane, M., Baudet, V., Boissin, P., Boursier, E. & Loriolle, F., et al. (1991) The bisindolylmaleimide GF 109203X is a potent and selective inhibitor of protein kinase C. *J Biol Chem*. Vol.266, No.24, pp.15771, ISSN 0021-9258

Vantelon, N., Rioux-Bilan, A., Ingrand, S., Pain, S., Page, G., Guillard, O., Barrier, L., Piriou, A. & Fauconneau, B. (2007) Regulation of initiation factors controlling protein synthesis on cultured astrocytes in lactic acid-induced stress. *Eur J Neurosci.* Vol.26, No.3, pp.689, ISSN 0953-816X

Vattem, M. & Wek, C. (2004) Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. *Proc Natl Acad Sci U S A*. Vol.101, No.31 pp11269, pp.74, ISSN 0027-8424

Vaupel, P., Kallinowski, F. & Okunieff, P. (1989) Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors. *Cancer Res.* Vol.49, pp. 6449, ISSN 0008-5472

Vogt, A., Sun, J., Qian, Y., Hamilton, D. & Sebti, M. (1997) The geranylgeranyltransferase-I inhibitor GGTI-298 arrests human tumor cells in G0/G1 and induces p21(WAF1/CIP1/SDI1) in a p53-independent manner. *J Biol Chem*. Vol.272, No.43, pp.27224, ISSN 0021-9258

Vukovic, V. & Tannock, IF. (1997) Influence of low pH on cytotoxicity of paclitaxel, mitoxantrone and topotecan. *Br J Csancer.* Vol.75, No.8, pp.1167, ISSN 0007-0920

Vujcic, S., Halmekyto, M., Diegelman, P., Gan, G., Kramer, L., Janne, J. & Porter, W. (2000) Effects of conditional overexpression of spermidine/spermine N1-acetyltransferase on polyamine pool dynamics, cell growth, and sensitivity to polyamine analogs. *J. Biol. Chem*. Vol.275, No.49, pp.38319, ISSN 0021-9528

Wang, Y., Xiao, L., Thiagalingam, A., Nelkin, D. & Casero, A. (1998) The identification of a cis-element and a trans-acting factor involved in the response to polyamines and polyamine analogues in the regulation of the human spermidine/spermine N1-

acetyltransferase gene transcription. *J. Biol. Chem.* Vol.273, No.51, pp34623, ISSN 0021-9258

Wang, K., Lin-Shiau, Y. & Lin, K. (2000) Suppression of invasion and MMP-9 expression in NIH 3T3 and v-H-Ras 3T3 fibroblasts by lovastatin through inhibition of ras isoprenylation. *Oncology.* Vol.59, No.3, pp.245, 0030-2414

Wang, J., Kitajima, I. (2007) Pitavastatin inactivates NF-kappaB and decreases IL-6 production through Rho kinase pathway in MCF-7 cells. *Oncol Rep.* Vol.17, No.5, pp.1149, ISSN 1523-3790

Warburg,O. (1956) On the origin of cancer cells. *Science.* Vol. 123, pp. 309, ISSN 0036-8075

Wei, J., Zhao, J., Long, M., Han, Y., Wang, X., Lin, F., Ren, J., He, T. & Zhang H. (2010) p21WAF1/CIP1 gene transcriptional activation exerts cell growth inhibition and enhances chemosensitivity to cisplatin in lung carcinoma cell. *BMC Cancer*. Vol.10, pp.632, ISSN 1471-2407

Wek, C., Jiang, Y. & Anthony, G. (2006) Coping with stress: eIF2 kinases and translational control. *Biochem Soc Trans*. Vol.34, No.7, No.1, pp.11, ISSN 0300-5127

Williams, J., Cowen, L. & Stratford, J. (2001) Hypoxia and oxidative stress. Tumour hypoxia--therapeutic considerations. *Breast Cancer Res.* Vol.3, No.5, pp.328, ISSN 0167-6806

Wu, H., Jiang, H., Lu, D., Xiong, Y., Qu, C., Zhou, D., Mahmood, A. & Chopp, M. (2009) Effect of simvastatin on glioma cell proliferation, migration, and apoptosis. *Neurosurgery.* Vol.65, No.6, pp.1087, ISSN 0148-396X

Yu, W., Li, K., Pang, K., Au-Yeung, C. & Ho, P. (2006) Anticancer activity of a series of platinum complexes integrating demethylcantharidin with isomers of 1,2-diaminocyclohexane. *Bioorg Med Chem Lett.* Vol.16, No.6, pp.1686, ISSN 0960-894X

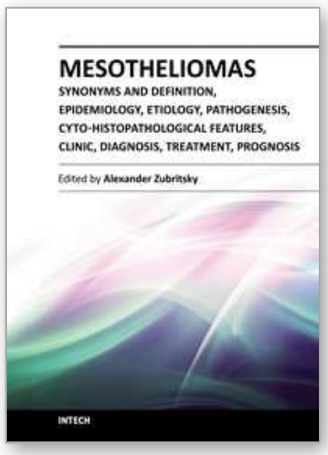
Zhang, C., Peng, Y., Wang, F., Tan, X., Liu, N., Fan, S., Wang, D., Zhang, L., Liu, D., Wang, T., Wang, S., Zhou, Y., Su, Y., Cheng, T., Zhuang, Z. & Shi, C. (2010) A synthetic cantharidin analog for the enhancement of doxorubicin suppression of stem cell-derived aggressive sarcoma. *Biomaterials.* Vol.31, No.36, pp.9535, ISSN 0142-9612

Zhao, TT., Trinh, D., Addison, CL. & Dimitroulakos, J. (2010) Lovastatin inhibits VEGFR and AKT activation: synergistic cytotoxicity in combination with VEGFR inhibitors. *PLoS One*. Vol.5, No.9, pp.e12563, ISSN 1932-6203

Zhou, D., Pallam, R., Jiang, L., Narasimhan, J., Staschke, A. & Wek, C. (2008) Phosphorylation of eIF2 directs ATF5 translational control in response to diverse stress conditions. *J Biol Chem*. Vol.283, No.11, pp7064, ISSN 0021-9258

Zorbas-Seifried, S., Hartinger, CG., Meelich, K., Galanski, M., Keppler, BK. & Zorbas, H. (2006) DNA interactions of pH-sensitive, antitumor bis (aminoalcohol) - dichloroplatinum(II) complexes. *Biochemistry.* Vol.45, No.49, pp.14817, ISSN 0006-2960

Zucali, P.A. & Giaccone, G. (2006) Biology and management of malignant pleural mesothelioma. *Eur. J. Cancer.* Vol. 42, pp.2706, ISSN 0959-8049



Mesotheliomas - Synonyms and Definition, Epidemiology, Etiology, Pathogenesis, Cyto-Histopathological Features, Clinic, Diagnosis, Treatment, Prognosis

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Mesotheliomas are mysterious mesothelial tumors in that they are relatively rare, difficult to diagnose, with a large number of synonyms, and the etiology and pathogenesis of the disease are still not fully disclosed. This problem attracts the attention of various specialists in the field of medicine and biology every year. In recent years there has been a significant increase of mesothelioma morbidity in most of the countries, due to the further industrialization of society. In this regard, this book has been published with the participation of an international group of experts with rich experience from around the world . The book consists of 14 chapters containing the most advanced achievements of all aspects of the various types of mesotheliomas, both in humans and domestic animals, at a high methodological level. This book is intended for biologists and all health care workers, mostly oncologists of different profiles, as well as students of medical educational institutions engaged or even just interested in the problems of mesotheliomas.

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