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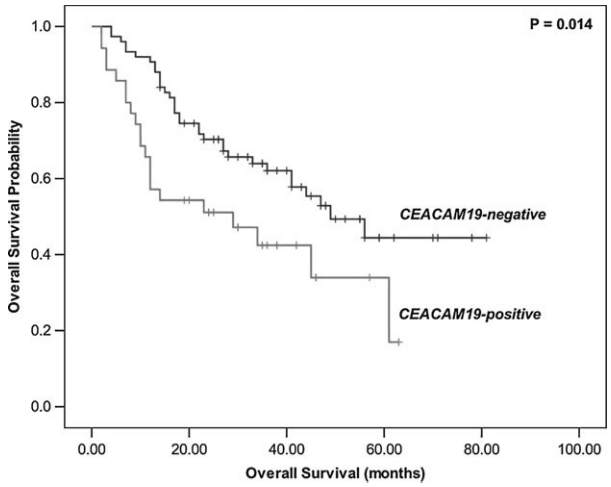

Fig. 1. Kaplan–Meier OS curves for CEACAM19 expression in NSCLC patients.

levels were quantified *via* an optimized qPCR method. SPSS Statistics software was used for biostatistical analyses.

CEACAM19 mRNA levels were slightly elevated in poorly differentiated compared to well differentiated tumors. Most importantly, patients stratified as *CEACAM19*-postitive exhibited inferior overall survival (OS) intervals compared to *CEACAM19*-negative ones (P = 0.014). The 5-year OS probability was 17.0% for *CEACAM19*-postitive patients and 49.3% for *CEACAM19*-negative ones. Univariate logistic regression corroborated the prognostic relevance of *CEACAM19* expression (HR = 1.96, 95% CI = 1.13–3.40, P = 0.016). Multivariate logistic regression analysis, adjusted for important clinicopathological parameters, including TNM stage, histotype, chemotherapy/radiotherapy administration, identified *CEACAM19* mRNA levels as a novel independent biomarker of poor prognosis for NSCLC patients (HR = 2.07, 95% CI = 1.11– 3.87, P = 0.023). The prognostic significance of *CEACAM19* was retained in the subgroup of low-risk patients (P = 0.041).

Improved risk stratification procedures are urgently needed for NSCLC. *CEACAM19* can aid in the decision-making for the management of NSCLC patients, since it has been identified by our study a biomarker that can provide important prognostic information which is independent of the currently used conventional indicators such as the TNM staging system.

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Keywords: Biomarker, CEACAM19, lung cancer.

TUE-040
Cell lineage-dependent activation of NF-κB by human T-cell leukemia virus type 1 Tax1

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Human T cell leukemia virus type1 (HTLV-1) is shown to be a causative agent of adult T cell leukemia (ATL), an aggressive malignancy. HTLV-1 produces the transcriptional modulator Tax1. Tax1 exerts its activities through activation of cellular transcription factors, among which NF-kB is demonstrated to be important for T cell transformation. There are two sub-pathways in the NF-kB system, canonical and non-canonical pathways. Though both pathways have been demonstrated to be activated by Tax1, we found in this study that Tax differentially activated NF-kB sub-pathways in a cell lineage-dependent manner.

Luciferase reporter plasmids with an immunoglobulin gene NF-kB binding element (IgkB site) and an OX40 ligand gene NF-kB binding element (gpkB site) were used to monitor NF-kB activities in response to Tax1. Expression of Tax1 activated IgkB and gpkB sites in hematopoietic cell lines such as Jurkat (human T cell leukemia cell line) and K562 (human erythroleukemia cell line). Interestingly the gpkB site was not activated by Tax1 in non-hematopoietic cell lines such as MG63 (human osteosarcoma cell line) and REF56 (rat embryonic fibroblast cell line) unlike the IgkB site, and exogenous introduction of RelA activated the gpkB site in non-hematopoietic cell lines. We found that hematopoietic cell lines activated the canonical and non-canonical pathways of NF-kB in response to Tax1, while only the non-canonical pathway was activated by Tax1 in non-hematopoietic cell lines. As TNF alpha stimulated NF-kB in non-hematopoietic cell lines, the canonical pathway system was functional in those cells. Tax1 however did not induce translocation of RelA to the nucleus, resulting in failure in RelA binding to the gpkB site. Chromatin immunoprecipitation assays revealed that non-canonical pathway components RelB and p52 bound the IgkB site, but the gpkB site showed p50 binding only in those cells. These results indicate that the gpkB site may be preferentially activated by the canonical pathway at least in hematopoietic cell lines tested. Importantly HTLV-1 Tax1 activates NF-kB in more restricted conditions than we thought, presumably accounting for pathogenesis of HTLV-1 infection.

Keywords: HTLV-1, leukemia, NF-kB.

TUE-041
Cellular internalization of Bortezomib loaded CS MNPs by cervical cancer cells

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Use of nanotechnology in cancer treatment offers exciting opportunities, including the possibility of destroying tumors with minimal damage to healthy tissue by novel targeted drug delivery systems. Chitosan coated superparamagnetic iron oxide nanoparticles (CS MNPs) were *in-situ* synthesized by ionic crosslinking

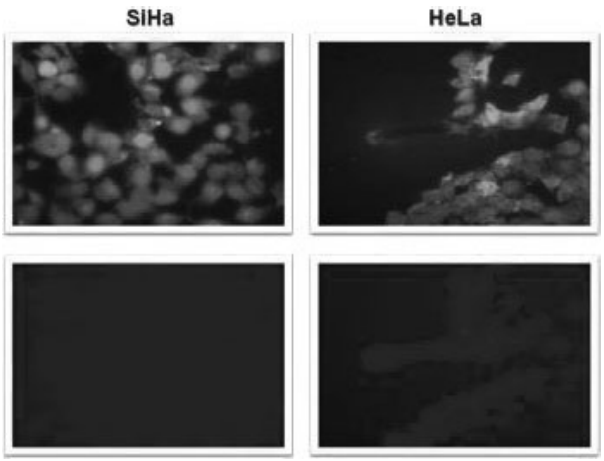

Fig. 1. Cellular internalization and localization of FITC labeled Bortezomib loaded CS MNPs on SiHa and HeLa cell lines.

method as nanocarrier systems (Unsoy *et al.*, 2012) and loaded with the anti-cancer drug Bortezomib (Velcade®).

Bortezomib loaded CS MNPs were labeled with FITC and visualized inside the cells by fluorescence microscopy on DAPI stained cervical cancer cell lines (SiHa and HeLa). Internalization of drug loaded nanoparticles can be clearly identified by the intensity of fluorescent color in the cells. FITC bounded Bortezomib loaded CS MNPs are successfully taken up by the cells and localized both in the cytoplasm and the nuclei of the cells (Figure 1).

Therefore, synthesized and Bortezomib loaded CS MNPs can be effectively enter to the cells and show its antiproliferative activity by inhibiting proteasome on cervical cancer cell lines.

Keywords: Bortezomib, cervical cancer, nanoparticle, FITC.

TUE-042
Cellular internalization of polyhydroxybutyrate coated magnetic nanoparticles in SKBR-3 cell lines

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Biodegradable polymeric nanomaterials gained importance in biomedical and bioengineering research such as drug delivery and targeting, tissue engineering, cancer diagnosis and therapy. Polyhydroxybutyrate (PHB) is a nontoxic, biodegradable, biocompatible polymer, and hence is suitable for medical applications. In this study, PHB coated magnetic nanoparticles (PHB-MNPs), were produced for targeted delivery of anticancer agent in cancer chemotherapy. PHB-MNPs were synthesized by in situ precipitation method. PHB-MNPs were incubated with breast cancer (SKBR-3) cell lines in 6 well plates and their photographs were taken with time intervals during the incubation to determine their cellular internalization. The cellular internalization of PHB-MNPs was demonstrated by fluorescence microscopy. It was revealed that these nanoparticles are efficiently internalized by the SKBR cells, and seem to be suitable for biomedical applications.

Keywords: PHB, magnetic nanoparticles, cellular internalization, SKBR-3 cell line.

TUE-043
Characterization of 7-dehydrocholesterol cytotoxic effects on melanoma cell lines

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Ultraviolet radiation is the main cause of skin cancers, and melanoma is the most serious form of tumor. Surgery is standard treatment for localized melanoma, while there is no therapy for advanced-stage of melanoma and its metastasis, because of the high resistance of melanoma cells to various anticancer therapies. Human skin is an important metabolic organ in which occurs photo-induced synthesis of vitamin D3 from 7-dehydrocholesterol (7-DHC). The 7-DHC, the precursor of cholesterol biosynthesis, is highly reactive and easily modifiable to produce 7-DHC-derived compounds. The intracellular levels of 7-DHC or its derivatives can have deleterious effects on cellular functionality and viability.

In this study we evaluated the effect on melanoma cell lines by 7-DHC as such and for this aim much care to minimize 7-DHC modifications was used. We found that from 12 to 72 hours of treatment 82-86% of 7-DHC entered into the cells, and the levels of 7-DHC-derivative compounds were not significant. At same time ROS production was significantly increased already after 2 hours and, after 24 hours, a reduction of cell viability was observed. Indeed, after 48-72 hours a pro-apoptotic effect of 7-DHC was detected. The cytotoxic effect of 7-DHC was associated with an increase in Bax levels, decrease in Bcl-2/Bax ratio, reduction of mitochondrial membrane potential, increase in apoptosis-inducing factor levels, unchanged caspase-3 activity, and absence of cleavage of PARP- 1. These findings could explain the mechanism through which 7-DHC exerts its cytotoxic effects. This is the first report in which the biological effects found in melanoma cells are mainly attributable to 7-DHC as such.

Keywords: 7-dehydrocholesterol, Melanoma cells, Reactive Oxygen Species.

TUE-044
Characterization of 7-dehydrocholesterol cytotoxic effects on melanoma cell lines

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Ultraviolet radiation is the main cause of skin cancers, and melanoma is the most serious form of tumor. Surgery is standard treatment for localized melanoma, while there is no therapy for advanced-stage of melanoma and its metastasis, because of the high resistance of melanoma cells to various anticancer therapies. Human skin is an important metabolic organ in which occurs photo-induced synthesis of vitamin D3 from 7-dehydrocholesterol (7-DHC). The 7-DHC, the precursor of cholesterol biosynthesis, is highly reactive and easily modifiable to produce 7-DHC-derived compounds. The intracellular levels of 7-DHC or its derivatives can have deleterious effects on cellular functionality and viability.

In this study we evaluated the effect on melanoma cell lines by 7-DHC as such and for this aim much care to minimize 7-DHC modifications was used. We found that from 12 to 72 hours of treatment 82-86% of 7-DHC entered into the cells, and the levels of 7-DHC-derivative compounds were not significant. At same time ROS production was significantly increased already after 2 hours and, after 24 hours, a reduction of cell viability was observed. Indeed, after 48-72 hours a pro-apoptotic effect of 7-DHC was detected. The cytotoxic effect of 7-DHC was associated with an increase in Bax levels, decrease in Bcl-2/Bax ratio, reduction of mitochondrial membrane potential, increase in apoptosis-inducing factor levels, unchanged caspase-3 activity, and absence of cleavage of PARP- 1. These findings could partially explain the mechanism through which 7-DHC exerts its cytotoxic effects. This is the first report in which the biological effects found in melanoma cells are mainly attributable to 7-DHC as such.

Keywords: 7-dehydrocholesterol, Melanoma cells, Reactive Oxygen Species.