

CD81 on MM-PC surface in around half of myeloma cases. Accordingly, we found a significant correlation between GEP and MFC expression of CD81 ($r = .743$; $P < 0.001$), and CD81+ SMM and MM patients showed higher levels of relative expression of CD81 mRNA compared to CD81- cases (7.5 vs. 6.6, $P = .03$ and 7.4 vs. 6.7, $P = .04$, respectively). No significant differences were found in baseline characteristics of CD81- vs. CD81+ SMM or MM patients, except for the % of MM-PC in S-phase (0.8 vs. 1.4, $P = .09$ and 0.9 vs. 1.4, $P = .003$ for SMM and MM, respectively). Finally, CD81+ SMM patients had a shorter time to progression to symptomatic disease than CD81- cases (median not reached – NR – vs. 25 months, $P = .04$); and also CD81+ MM showed significantly lower response rates (complete remission: 8% vs. 26%, $P = .01$), progression-free (median 23 vs. NR months, $P = .001$) and overall survival ($P = .03$) than CD81- cases.

Conclusions: Our findings uncover the existence of a phenotypic/genomic correlation of CD81 expression in MM-PC, which correlated with an adverse outcome in SMM and MM, supporting the clinical relevance of baseline routine BM evaluation by MFC in myeloma.

[596] The effect of cabbage juice and its active components on the protein level and expression of CYP1A1, CYP1A2 and CYP1B1 in MDA-MB-231 breast cancer cells

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Breast cancer is the most common malignancy in women. A major effort to reduce breast cancer mortality and morbidity is focused on development of better breast cancer chemoprevention. Although pharmaceutical agents have therapeutic and preventive roles in breast cancer, the use of compounds from natural products to prevent breast cancer is currently being explored. Among the promising food components being investigated to reduce breast cancer risk are phytochemicals found in cruciferous vegetables – indole-3-carbinol (I3C), diindolylmethane (DIM) and sulforaphane (SUL). In addition epidemiological studies have indicated that high intake of white cabbage may be associated with a lower risk of neoplastic diseases such as cancer of breast.

Our previous study showed that cabbage juice and its potential active components affected the proteins level and expression of CYP450 isoenzymes involved in estrogen biosynthesis and metabolism in breast cancer estrogen dependent (MCF7) and human epithelial nontumorigenic (MCF10A) cell lines.

The aim of the present study was to determine the effect of cabbage juices, I3C, DIM and SUL on the expression profile of CYP1A1, CYP1A2 and CYP1B1 mRNA and proteins level in breast cancer estrogen independent (MDA-MB-231) cell line. Cells were treated with raw cabbage and sauerkraut juices (obtained from vegetables cultivated in industrial and ecological farms) or I3C, DIM and SUL at the concentrations relevant to those observed in human plasma. After 72 hours of incubation the screening of cDNA from total RNA was performed using real-time PCR assay with specific primers for CYPs and protein level was determined by Western blot analysis.

The most marked effect was observed on the mRNA level. The enhanced expression of CYP1A1 and CYP1B1 was noticed as a result of treatment of MDA-MB-231 cells with both doses of I3C and DIM. CYP1B1 mRNA level was also increased by all tested cabbage juices. In case of CYP1A1 expression the similar effect was observed after treatment with juices obtained from vegetables cultivated in ecological farms.

The results of this study provide additional data on the possible anticarcinogenic and antimutagenic activity of cabbage.

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[597] Modulation of transcription factor Nrf2 as mechanism of chemoprotective effects of cabbage juices, indole-3-carbinol and phenethyl isothiocyanate in human hepatoma cells and rat liver

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Epidemiological studies suggest that the intake of Brassica vegetables is associated with a decreased risk of developing cancer. This effect is usually linked to the intake of glucosinolates and their metabolism to cancer preventive indoles, such as indole-3-carbinol (I3C) and isothiocyanates, such as phenethyl isothiocyanate (PEITC). These compounds lead to the induction of phase 2 enzymes of xenobiotic metabolism. In the last few years it was shown that the main role in the induction of phase 2 enzymes is played by the Nrf2 transcription factor. Nrf2 activates various genes encoding cytoprotective proteins which deactivate reactive electrophilic metabolites of xenobiotics, decompose reactive oxygen species and stabilize the cell redox potential. Our previous studies demonstrated that the administration of cabbage or

sauerkraut juice increases the activity of glutathione transferase (GST) and changes the level of expression of its isoenzymes in rat liver.

In order to explain the mechanism of GST induction in this study the activation of Nrf2 was evaluated in rat liver and compared with the effect of cabbage and sauerkraut juices and I3C and PEITC in human hepatoma cells (HepG2). Male Wistar rats were treated by gavage with cabbage juices, I3C or PEITC for 4, 10 and 30 days. The HepG2 cells were incubated with cabbage juices, I3C or PEITC for 24 hours and Nrf2 levels were assessed by immunoblotting.

The treatment with either cabbage juices or pure compounds (I3C and PEITC) resulted in the translocation of Nrf2 protein from cytosol to the nucleus in both the *in vivo* and *in vitro* model. Western blot analysis showed the most significant enhancement of nuclear Nrf2 levels in rat liver after 30 days of treatment with cabbage juice, I3C or PEITC. Cabbage juices and other tested compounds showed a similar effect in HepG2, but the highest level of Nrf2 in the nuclear fraction was observed in cells incubated with PEITC.

The results of our present and earlier studies indicate that the induction of GST through the activation of Nrf2 by cabbage juices may be responsible for their chemopreventive activity demonstrated by epidemiological studies and in animal models. However, the activation of Nrf2 in cancer cells may enhance resistance to chemotherapeutic drugs and should be taken into consideration in dietary recommendations for cancer patients.

[598] DNA cancer vaccine mediated by polyethylenimine

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Background: Polycations have been widely used in non-viral gene delivery. This study attempts to investigate the adjuvant effect of polyethylenimine (PEI) on stimulation of antigen-specific immune response via DNA-based cancer vaccine.

Materials and Methods: Plasmid DNA encoding the ovalbumin (OVA) gene was complexed with polyethylenimine (PEI) and subcutaneously injected into the C57BL/6J mice. Cells were isolated from the secondary lymphoid organs and assayed by an OVA/K^b-specific murine cytolytic T-hybridoma. Specific lysis of the target cells was studied employing the *in vivo* cytotoxic T-lymphocyte (CTL) assay by labeling the target cells with CFSE (carboxyfluorescein succinimidyl ester), and the effect of PEI-mediated DNA vaccination on tumour growth was examined in the EL4 and EG7-OVA thymoma model. Both tumour volumes and the animal survival rates were routinely monitored. Animal care and experimental treatment were conducted in compliance with the institutional policies.

Results: Treatment of animals with the PEI-DNA complexes resulted in significant activation of an OVA-specific cytotoxic clone that recognizes the target cells through the class I major histocompatibility complex (MHC) molecules, illustrating the induction of class I-restricted antigen presentation *in vivo*. Antigen-specific cell lysis was demonstrated by the CFSE-based *in vivo* assays. Immunohistochemical staining showed that adjuvant-mediated DNA vaccination induced cell death and significant lymphocyte infiltration at the injection sites. Vaccination of the C57BL/6J mice with plasmid DNA/PEI complexes, either preceded or after the tumour challenges, lead to suppression of the tumour growth and prolonged the survival rate of the animals.

Conclusions: Our data illustrated the potential use of polycations in DNA-based cancer therapy for induction of antigen-specific immune response and CTL effect, resulting in the protective and therapeutic immunity in the experimental tumour models.

[599] C-reactive protein; a potential marker of second cancer and cardiovascular disease in testicular cancer survivors?

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Purpose: C-reactive protein (CRP) is a marker of cardiovascular risk both in patients with cardiovascular disease (CVD) and in presumably healthy patients with normal LDL cholesterol while there is a conflicting evidence regarding CRP as a marker of future cancer. The aim was to assess whether CRP predicts CVD and consecutive cancer in testicular cancer survivors (TCSS).

Methods: During 1998–2001, 586 TCSS with a high sensitivity CRP ≤ 10 mg/L were identified median 11 (4–21) years after treatment (FU-1). A second follow-up survey (FU-2) was conducted median 8 (6–9) years after FU-1. At FU-2 we obtained information about post-FU-1 CVD (cardiovascular death, nonfatal myocardial infarction, stroke, revascularization or heart failure). Information about post-FU-1 non-germ cell cancer and cardiovascular death in all 622 patients were retrieved from the Cancer Registry of Norway.

Results: After FU-1 31 (5.3%) of 586 patients developed non-germ cell cancer (excluding localized prostate cancer) while 28 (4.9%) developed CVD.