

**Conclusions:** Statins appear to be protective against the development of pancreatic cancer and the magnitude of the effect correlates with the duration of statin usage.

### P37

#### **Circumvention of DNA methylation and histone deacetylation causes re-expression of TSGs leading to apoptosis of breast Ca and chemoprevention of subsequent metastasis**

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In this study, we prove that DNA methylation is very important in the chemoprevention of metastasis of breast cancer. Generally, promoter hypermethylation is an epigenetic alteration which causes repression of gene transcription in breast cancer cells. We examine tumor suppressor genes, candidate tumor suppressor genes and other genes involved in transcription factors, cell differentiation, apoptosis, growth inhibitory signals, metabolism, antiproliferative signals, replicative senescence, angiogenesis, cell adhesion, tissue invasion, metastasis and other important regulatory proteins which have been transcriptionally inactivated due to aberrant methylation of promoter region CpG islands. We obtain metastatic tumour cells from breast cancer patients and they were implanted in immunosuppressive animals. We obtained DNA from serum, ductal lavage fluid and tumor cells excised by surgery from animals with implanted breast cells and we analysed the specimens with RLGS, MCA-RDA, MS-AP-PCR, DMH, Microarray and Gene re-expression analysis, Bisulfite sequencing, Southern blot, Immunoprecipitation, Western blot and IHC. We analyze the specimens before and after monotherapy with vinorelbine and combined regimen composed of demethylating agent hydralazine and Vinorelbine. Before treatment, we observed promoter hypermethylation and repression of transcription of the following genes: p16 (INK4a/CDKN2A), p73, p15 (INK4b/CDKN2B), p14ARF, CCND2, SFN, RARb2, HIN-1, BRCA1, GSTP1, FAPB3, HOXA5, p21WAF1/CIP1/SDI1, E-cadherin (CDH1), TIMP3, MGMT, APC, RASSF1A, NOEY2 (ARHI), RARb2, MDGI, P27KIP1, Gelsolin, 14-3-3 Sigma (Stratifin), Mad, HMLH1, Nm23-H1, 3-OST-2, Maspin, HIC1, MDG1 and Rb. Results remained stable after monotherapy with vinorelbine. In contrast, after combined treatment with Vinorelbine and hydralazine, we observed inhibition of DNA methylation resulting in activation of gene expression which was accompanied by acetylation of histones. This caused decondensation of chromatin allowing access to endogenous proapoptotic endonucleases. Furthermore, hydralazine re-expressed genes relevant for irreversible apoptotic cell death, type D2. Ki-67 exhibited inhibition of tumour proliferation and MTT assay exhibited inhibition of metabolic activity. TEM and SEM exhibited zeiosis, cytoplasmic and nuclear condensation leading to karyorexis with endolytic cleavage of the DNA into small oligonucleosomal fragments which were phagocytosed by macrophages and adjacent tumor cells. MRI exhibited eradication of tumor sites and no sign of metastatic sites. Concluding, the combined administration of Hydralazine and Vinorelbine is responsible for the re-expression of genes characterised by DNA methylation

and histone deacetylation leading to eradication of breast cancer cells and offering a promising new chemopreventive option against metastasis of breast cancer.

### P38

#### **Protection against UV-light-induced skin carcinogenesis by topical or dietary administration of broccoli sprout extracts as a source of sulforaphane in SKH-1 high-risk mice**

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The increase in skin cancer incidence is expected to persist due to ozone depletion, increased sun exposure, and longer life expectancy. The effects of UV light contributing to tumor formation are at least three types: (i) inflammation; (ii) direct DNA damage, and (iii) generation of reactive oxygen species. Phase 2 enzymes and glutathione protect against electrophiles and oxidants. Their induction is an efficient strategy for protection against cancer. Sulforaphane, an isothiocyanate isolated from broccoli guided by a bioassay for phase 2 inducer activity, is one of the most potent naturally occurring inducers known. The plant contains a precursor of sulforaphane, the glucosinolate glucoraphanin. Upon injury, glucoraphanin comes in contact with the otherwise compartmentalized myrosinase that catalyzes its hydrolysis to sulforaphane. We tested the hypothesis that topical or dietary administration of broccoli sprout extracts as a source of sulforaphane could protect against the development of skin tumors in mice that were rendered high-risk by chronic exposure to UV light (30 mJ/cm<sup>2</sup>/session twice a week for 20 weeks). Two extracts (equivalent to 0.3 mmol [low dose] or 1.0 mmol [high dose] sulforaphane, respectively) were applied topically to groups of 30 mice once a day, 5 days a week, for 11 weeks, at which time point all the control animals had tumors. There was a 50% reduction in tumor burden, incidence, and multiplicity in the animals receiving the high dose of protector. Tumor incidence and multiplicity did not differ between the low dose-treated and the control groups, however the low dose treatment provided a substantial reduction in the overall tumor burden. Two extracts (equivalent to 10 mmol/day [low dose] and 50 mmol/day [high dose] glucoraphanin) were incorporated in the diet and the animals were fed for 15 weeks, at which time point 93% of the control mice had tumors. Tumor incidence was reduced by 25% and 35% in the animals receiving the low dose and the high dose of glucoraphanin, respectively. Even greater was the effect of treatment on tumor multiplicity which was reduced by 47% and 72%, respectively: Thus, while the animals in the control group had on the average of 4.3 tumors per mouse, the number of tumors per mouse was 2.3 for the low dose and 1.2 for the high