BELGIAN ASSOCIATION FOR THE STUDY OF CANCER

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Plasminogen activator inhibitor type I (PAI-1) controls bone marrow-dependent and independent vascularization.

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Abstract :
Plasminogen Activator Inhibitor-1 (PAI-1) deficiency in mice is associated with impaired choroidal neo-vascularization (CNV) and tumoral angiogenesis. We investigated whether bone marrow-derived (BM) cells contribute to these two neo-vascularization processes regulated by PAI-1. We show here that the cellular source of PAI-1 differs in these pathological processes. *PAI-1*$^{-/-}$ mice grafted with BM-derived from Wild Type (WT) mice were able to support laser-induced CNV formation, but not skin carcinoma vascularization. Engraftment of irradiated WT mice with *PAI-1*$^{-/-}$ BM prevented CNV formation demonstrating the pivotal role of PAI-1 delivered by BM-derived cells. In sharp contrast, tumor vascularization is dependent to PAI-1 status of local resident cells, but not of BM cells. Differences between these two models could be related to the differential contribution of Placenta-like Growth Factor (PlGF) and Vascular Endothelial Growth Factor (VEGF), angiogenic factors involved in BM cell recruitment. Altogether, these data identify different cellular mechanisms contributing to PAI-1-regulated pathological neo-vascularization. They shed new lights on what role individual cells play in the pathogenesis of ocular disease and cancer.

Caveolin-1 plays key roles in the regulation of tumor angiogenesis: evidence for the activation of multiple pro-angiogenic signaling pathways in caveolin-deficient mice.

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Caveolae are 50- to 100-nm invaginated microdomains of the plasma membrane that function as regulators of signal transduction. Caveolin-1 belongs to a class of oligomeric structural proteins that are necessary for caveolae formation and has been involved in the regulation of several pathogenic processes, including tumor development. We have recently documented that caveolin-deficient mice exhibited a higher extent of tumor angiogenesis and we have therefore designed a series of experiments on more tractable models of angiogenesis to identify the determinants of this phenomenon.
Using an ex vivo angiogenesis model, in which aortic explants from caveolin-1 deficient mice were cultured in 3D collagen type I gels in the presence of autologous serum, we showed that the absence of caveolin-1 was associated with a significantly higher extent of tube formation (than from wild-type mouse explants) ($P<0.01$, $n=8$). This observation was reproduced in an in vitro model of angiogenesis based on the ability of endothelial cells to form network when cultured on Matrigel (mixture of extracellular matrices extracted from tumors). Interestingly, further investigations revealed that the observed pro-angiogenic activity in caveolin-deficient mice arose both from the serum and from intrinsic characteristics of the vascular tissue of these mice. Accordingly, we documented that caveolin-1 deficiency was associated with an increased activity of the endothelial nitric oxide synthase (eNOS) and the consecutive accumulation of circulating nitric oxide-derived products in the blood of these animals. We further provided evidence that these NO derivatives exerted direct angiogenic activities, particularly in situations where hypoxia and acidosis were present. As for the tissue-specific effects, we found that the lack of caveolin left the bFGF signaling pathway unopposed. This observation originally made in the aorta explant assay was confirmed with endothelial cells where the caveolin gene was down regulated using the siRNA technology or with endothelial cells directly derived from caveolin-deficient mice. Accordingly, caveolin deficiency was shown to promote ERK activation downstream bFGF stimulation and to prevent bFGF receptor internalization, thereby accounting for a constitutive activation of this signaling pathway.
In conclusion, this study has identified key roles played by caveolin in the regulation of angiogenesis. That the lack of caveolin leads to the activation of signaling pathways involving key pro-angiogenic actors such as nitric oxide and bFGF opens interesting therapeutic perspectives wherein the recombinant expression of caveolin could dampen tumor angiogenesis and thereby limits tumor growth.

Caveolin-1 is critical for the maturation of tumor blood vessels through the regulation of both endothelial tube formation and pericyte coverage.

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Extravasation of plasma proteins from tumor blood vessels accounts for the formation of a provisional matrix acting as a scaffold for proliferating and migrating endothelial cells during angiogenesis. In normal vascular beds, the structural protein caveolin directly participates in transcytosis (through caveolae-derived channel formation or shuttling vesicles) and in paracellular NO-mediated permeability (through the allosteric modulation of the endothelial nitric oxide synthase). Here, we used caveolin-deficient mice (cav-/-) and wild-type animals (cav+/+) to track the role of caveolin/caveolae in the regulation of tumor (B16 melanoma) blood vessel permeability.
We first used radio-iodinated bovine serum albumin injection to get a global view of microvascular permeability in tumor-bearing mice of either caveolin genotype (-/- and +/+). We found that in cav-/- mice, albumin was more rapidly cleared from the circulatory system and accumulated more extensively in skeletal muscles. However, in the tumors, no difference in albumin extravasation was observed according to the caveolin genotype. Tumor immunostaining for fibrinogen confirmed that the extent of macromolecule extravasation around small tumor blood vessels was not different in cav+/+ and cav-/- mice. Of note, however, more diffuse area of fibrinogen staining could be detected around large vessels in cav-/- mice. The wick-in-needle technique revealed a net increase in interstitial fluid pressure (IFP) in tumor-bearing cav-/- mice (17.4 ± 0.1 vs 11.0 ± 0.8 mmHg in cav+/+ mice; n=6). Search for immune cell infiltration or fibroblast proliferation in cav-/- mice, with CD11b and CD90 antibodies, respectively, did not lead to the identification of differences supporting the IFP increase. In cav+/+ mice, however, fibroblasts could be found in significantly larger amounts around blood vessels. This observation prompted us to examine the pericyte coverage of tumor blood vessels with an antibody directed against alpha-smooth muscle actin (SMA). Interestingly, while in cav+/+, we found a homogenous distribution of microvessels exhibiting the double CD31 and SMA staining across the tumor, cav-/- mice revealed two very distinct vessel populations. At the periphery, we found very large vessels whereas the centre of the tumor presented a very dense population of small microvessels. Moreover, in both vessel types, the pericyte coverage (SMA positive staining) was either absent or very limited, but in all cases, significantly less developed than in Cav+/+ tumors.
To further explore this apparent defect in vessel maturation, we examined the endothelial and the fibroblast-like/pericyte outgrowth from aorta rings issued from both cav+/+ and cav-/- mice. The linearity of endothelial tubes outgrown from the aorta rings allowed to discriminate the proportion of corresponding DAPI-stained nuclei from the more dispersed ones associated to fibroblast-like cells. Accordingly, we found that Cav-/- aorta rings gave rise to 1.5-fold more endothelial tubes than Cav+/+ mice (P<0.01) but that the number of fibroblast-like cells was dramatically reduced (13.3 ± 2.0 vs 25.4 ± 3.5 per microscopic field in Cav+/+; P<0.01, n=10). Altogether, these data indicate that caveolin regulates angiogenesis but also the recruitment of pericytes to neo-formed endothelial tubes. Caveolin appears therefore as an important control point for the maturation of tumor blood vessels and the regulation of IFP and can be viewed as a new target for diagnostic or therapeutic strategies.

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The extracellular fragment of N-cadherin stimulates angiogenesis and migration and invasion of cells

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A cell becomes cancerous as a result of essential alterations in its physiology: limitless replicative potential, induction of angiogenesis and acquisition of invasive and metastasizing potential. The processes of both angiogenesis and invasion consist of the following aspects: loss of cell-cell contacts, gain in proteolytic activity and increased directional migration. The cadherins, transmembrane calcium-dependent adhesion molecules, play an important role in these processes. In a number of epithelial tumors the switch from E- (epithelial) to N- (neural) cadherin is a well described phenomenon. As a result the cells change from an epithelioid to a fibroblast-like phenotype and become more invasive. In the microenvironment of the cancer cells proteases are secreted which are responsible for the shedding of the extracellular domain of N-cadherin, named soluble N-cadherin. N-cadherin is also expressed by endothelial cells and pericytes and plays a role in angiogenesis. Soluble N-cadherin is still functional: it stimulates neurite outgrowth. However the possible influence of soluble N-cadherin on endothelial and cancer cell function and its signalling pathway are not well investigated.
RESULTS: We found that soluble N-cadherin is produced by all N-cadherin positive cells and that the cleavage could be stimulated by the use of plasmin, a serine protease. As a model for our experiments we are using sarcoma cells transfected or not with chicken N-cadherin, and human endothelial cells. Human soluble N-cadherin was purified and used to treat cells in different migration assays. We could prove that soluble N-cadherin stimulates the cancer cells to migrate in the wound healing assay and to invade in the collage type I assay. Soluble N-cadherin stimulates angiogenesis and the histidine-Alanine-Valine-(HAV) sequence is important for this effect. The stimulatory effect could be inhibited by using the fibroblast growth factor receptor (FGFR) inhibitor PD173074. Soluble N-cadherin interferes with the N-cadherin –FGFR complex and activates the mitogen activated protein kinase (MAPK) signalling pathway.
CONCLUSION: Soluble N-cadherin stimulates cancer cells to migrate and invade and stimulates angiogenesis.

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Tumor radiosensitization by anti-inflammatory drugs : evidence for a new mechanism involving the oxygen effect.

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We hypothesized that nonsteroidal anti-inflammatory drugs (NSAIDs) may enhance tumor radiosensitivity by increasing tumor oxygenation (pO_2), via either a decrease in the recruitment of macrophages or from inhibition of mitochondrial respiration. The effect of four NSAIDs (diclofenac, indomethacin, piroxicam, NS-398) on pO_2 was studied in murine TLT liver tumors and FSAII fibrosarcomas. At the time of maximum pO_2 (t_{max}, 30 min after administration), perfusion, oxygen consumption and radiation sensitivity were studied. Local pO_2 measurements were performed using electron paramagnetic resonance (EPR). Tumor perfusion and permeability measurements were assessed by dynamic contrast-enhanced magnetic resonance imaging. The oxygen consumption rate of tumor cells after in vivo NSAID administration was measured using high frequency EPR. Tumor-infiltrating macrophage localization was performed with immunohistochemistry using CD11b anti-body. All the NSAIDs tested caused a rapid increase in pO_2. At t_{max}, tumor perfusion decreased, indicating that the increase in pO_2 was not caused by an increase in oxygen supply. Also at t_{max}, global oxygen consumption decreased but the amount of tumor-infiltrating macrophages remained unchanged. Our study strongly indicates that the oxygen effect caused by NSAIDs is primarily mediated by an effect on mitochondrial respiration. When irradiation (18 Gy) was applied at t_{max}, the tumor radiosensitivity was enhanced (regrowth delay increased by a factor of 1.7). These results demonstrate the potential utility of an acute administration of NSAIDs for radiosensitizing tumors, and shed new light on the mechanisms of NSAID radiosensitization. These results also provide a new rationale for the treatment schedule when combining NSAIDs and radiotherapy.

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Reversal of temporal and spatial heterogeneities in tumor perfusion identifies the tumor vascular tone as a tunable parameter to improve drug delivery.

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The maturation of the tumor vasculature implies the recruitment of pericytes covering and protecting the endothelial tubes from a variety of stresses including anti-angiogenic drugs. Mural cells also provide mature tumor blood vessels with the ability to either relax or contract in response to substances present in the tumor microenvironment. The observed cyclic alterations in tumor blood flow (TBF) and the associated deficit in chemotherapeutic drug delivery could arise from the influence of such vasomodulators.
To challenge this hypothesis, we focused our work on endothelin-1 (ET-1) which, besides its largely ubiquitous, autocrine effects on tumor cell growth, is a powerful vasoconstrictor. We first documented that an ET_A receptor antagonist could induce the relaxation of microdissected tumor arterioles, and selectively and quantitatively increase TBF in experimental tumor models. We then combined dye staining of functional (perfused) vessels, fluorescent microspheres-based mapping and dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI) to identify TBF heterogeneities and examine the reversibility of such phenomenon. We found that administration of ET_A receptor antagonist could indeed acutely reduce the extent of underperfused tumor areas, thereby proving the key role of vessel tone variations in the TBF heterogeneities. Importantly, we also provided evidence that the ET_A antagonist administration could, despite an increase in tumor interstitial fluid pressure, improve the access of the contrast agent used in DCE-MRI and of conventional chemotherapy to the tumor compartment. We showed that the combinatory administration of cyclophosphamide with the ET_A antagonist led to a significant increase in tumor growth delay when using low doses of cyclophosphamide and even to the tumor control when higher doses were used (vs either treatment given separately).
In conclusion, we report here that tumor endogenous ET-1 production largely participates in the temporal and spatial variations in TBF and that ET_A antagonist administration may wipe out such heterogeneities, thereby eliciting an adjuvant strategy to improve the delivery of chemotherapeutic drugs to the tumor.

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Welk teken ontbreekt hier.
Wij kunnen dit niet
achterhalen!

Blood and lymph vessel invasion in breast cancer: a prospective histomorphometrical study in 101 patients

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Introduction: Blood (BVI) and lymph vessel invasion (LVI) are the histological correlates of the first steps of haematogenous and lymphatic metastasis in solid tumours. The discovery of new lymphatic endothelium specific markers such as D2-40, makes it possible to distinguish between blood and lymph vessels. Therefore, the aim of this study was to quantify and compare blood and lymph vessel invasion in a consecutive series of breast cancer patients.
Materials and Methods: Three consecutive 5 µm sections of all formalin-fixed paraffin embedded (FFPE) tissue blocks of 101 consecutive breast cancer resection specimens were (immuno)histochemically stained with haematoxylin-eosin and with antibodies against CD34 (pan-endothelium) and D2-40 (lymphatic endothelium). On every slide all vessels containing single tumour cells or tumour cell emboli were marked, and based on the immunohistochemical staining pattern, vascular invasion was assessed as LVI (CD34 or /D2-40) or BVI (CD34+/D2-40). LVI and BVI were assessed as intra- (being in contact with tumour cells or desmoplastic stroma) or peritumoral. The number of intra- and peritumoral vessels with LVI and BVI per patient was counted as well as the number of tumour cells in every vessel. Results were correlated with clinico-pathological variables, the growth pattern of the tumour and the presence of a fibrotic focus.
Results: In total 3390 (intra: 718, peri: 2672) vessels with LVI and 143 (intra: 80, peri 63) vessels with BVI were detected. The median number of FFPE blocks investigated per patient was 4. The number of patients with LVI was higher then the number of patients with BVI (LVI: 70.3% (intratumoral: 33.7% and peritumoral: 61.4%); BVI: 38.6% (intratumoral: 28.7% and peritumoral: 25.7%)). The absence/presence of BVI and LVI was concordant with the absence/presence of vascular invasion determined on the haematoxylin-eosin slides in 63.4% (p=0.002) and 77.2% (p<0.001) of cases, respectively. An association was found between the presence of intratumoral LVI (p=0.04) or peritumoral LVI (p=0.001) and axillary lymph node involvement. For BVI no association was found with axillary lymp node status.
Intra- and peritumoral BVI were strongly associated (concordance: 77.2% ,p<0.001), while for intra- and peritumoral LVI only a trend was found (concordance: 54.5%, p=0.08). LVI was associated with BVI, intratumorally (p=0.02), not peritumorally (p=0.16). Furthermore, more intratumoral LVI (p=0.05) and BVI (p=0.02) were found in tumours with an expansive growth pattern; more intra- and peritumoral LVI and BVI (p<0.05) were found in high grade tumors and tumors with a fibrotic focus. The size of the intravascular tumor embolus was larger in peritumoral then in intratumoral vessels (p<0.001), both for LVI and BVI.
Conclusion: Our data demonstrate that it is possible to reliably distinguish BVI and LVI in breast cancer resection specimens using recently characterized specific lymphatic endothelium markers. This is important to study the contribution of both processes to the metastatic cascade in breast cancer. Furthermore, our data sustain the hypothesis that haematogenous and lymphatic metastasis are specific but complementary pathways.

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Impact of VEGF-dependent angiogenesis on the lymphocyte-endothelium interactions: identification of nitric oxide as a control point of endothelial cell anergy.

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Cytolytic T lymphocytes play crucial roles in host defense against tumours. Accordingly, several studies have shown that leukocyte infiltration can positively influence the prognosis of cancer. However it is also known that tumour cells may evade or resist immune responses at multiple levels within the effector-target interaction. In this study, we postulated that one mode of resistance could be a defect in the leukocyte-vessel wall interactions. The nature of the endothelial cells lining tumor blood vessels is, indeed, different from the endothelium of healthy tissues: tumor angiogenesis implies for instance that endothelial cells are either proliferating or at least are under the influence of many cytokines while endothelial cells are largely quiescent in mature vascular beds of healthy organs.
To mimick the impact of the tumor microenvironment on the endothelium, we have exposed endothelial cells to both the cytokine TNF-a and the pro-angiogenic factor VEGF. We have then examined the ability of lymphoid Jurkat cells or freshly isolated human CD8 to adhere to the "activated" endothelial cells. A dramatic inhibition of adhesion was observed with both cell populations (tracked by pre-loading them with calcein as a fluorophore). Interestingly, short (30 min) and long (16h) VEGF exposures led to a similar reduction in the adhesion of immune cells. Although RT-PCR analyses revealed a slight decrease in the mRNA expression of major adhesion molecules including selectins and ICAM, the expression of these adhesion molecules at the cell surface was not significantly altered. Immunocytochemistry experiments eventually revealed that VEGF impaired the clustering of these adhesion molecules. Caveolae are plasmalemmal invaginations that have been proposed to participate in the concentration of adhesion molecules and we therefore examined the impact of modifying the abundance of caveolin (the structural protein of caveolae) on the ability of VEGF to alter the adhesion process. Accordingly, overexpression of caveolin-1 in endothelial cells could overcome VEGF-mediated inhibition of adhesion whereas transfection of siRNA directed against caveolin-1 could reduce the TNF-a-dependent increase in adhesion. Still, we failed to document a co-localization of caveolin-1 and adhesion molecules such as ICAM in endothelial cells, thereby excluding a role of caveolae per se in the clustering process. Instead, we identified the NO release by the endothelial NO synthase (eNOS) as the main target of altering caveolin abundance (eg, an increase in caveolin reducing the NO production and inversely). We confirmed this observation by using the NOS inhibitor L-NAME that reversed the inhibitory effects of VEGF on lymphocyte adhesion, thereby recapitulating the effects of caveolin overexpression. We also found that the NO-mediated rearrangement of the cytoskeleton could directly account for the VEGF-induced alteration in the adhesion molecules clustering process needed to ensure efficient lymphocyte recruitment.
In conclusion, this study provides new insights on the mechanisms leading to the anergy of endothelial cells in tumor blood vessels. Our work also opens new perspectives for anti-angiogenic strategies that could act as adjuvant approaches to immunotherapy.

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Botulinum Toxin potentiates cancer radiotherapy and chemotherapy

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Structural and functional abnormalities in the tumor vascular network are considered factors of resistance of solid tumors to cytotoxic treatments. To increase the efficacy of anti-cancer treatments, efforts must be made to find new strategies for transiently opening the tumor vascular bed in order to alleviate tumor hypoxia (source of resistance to radiotherapy) and improve the delivery of chemotherapeutic agents. We hypothesized that *Botulinum Neurotoxin* type A (BoNT-A) could interfere with neurotransmitter release at the perivascular sympathetic varicosities, leading to inhibition of the neurogenic contractions of tumor vessels and therefore improving tumor perfusion and oxygenation.
To test this hypothesis, BoNT-A was injected locally into mouse tumors (fibrosarcoma FSaII, hepatocarcinoma TLT) and electron paramagnetic resonance (EPR) oximetry was used to monitor pO_2 in vivo repeatedly for four days. Additionally, contrast-enhanced magnetic resonance imaging (MRI) was used to measure tumor perfusion in vivo. Finally, isolated arteries were mounted in wire-myograph to monitor specifically the neurogenic tone developed by arterioles that were co-opted by the surrounding growing tumor cells.
Using these tumor models, we demonstrated that local administration of BoNT-A (2 sites, dose 29 $U.kg^{-1}$) substantially increases tumor oxygenation and perfusion, leading to a substantial improvement in the tumor response to radiotherapy (20 Gy of 250 kV RX) and chemotherapy (cyclophosphamide 50mg/kg). This observed therapeutic gain results from an opening of the tumor vascular bed by BoNT-A, since we demonstrated that BoNT-A could inhibit neurogenic tone in the tumor vasculature.
In conclusion, the opening of the vascular bed induced by BoNT-A offers a way to significantly increase the response of tumors to radiotherapy and chemotherapy.

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Identification of HIF-1 independent proteins overexpressed under hypoxia in tumor cells.

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Our work takes place in the global effort that aims to have a better knowledge of the mechanisms by which tumor cells can adapt to low oxygen tension during cancer development. It has been shown that a hypoxic area rapidly develops in the center of a tumor due to the reject of the preexisting blood vessels to the peripheric area of the growing tumor mass. This hypoxic area is responsible for biochemical modifications within tumor cells that allow them not only to adapt to hypoxia, but also render them more resistant to chimio or radiotherapy treatments. The master regulator of this adaptive response is HIF-1 (Hypoxia Inducible Factor-1), which controls 60% to 80% of the genes that allow adaptation to hypoxia. HIF-1 is a heterodimeric transcription factor made up of two subunits, HIF-1α and ARNT (Aryl Receptor Nuclear Translocator). However, if HIF-1 is important for the adaptive response to hypoxia, it does not explain all the complexity of this response and the other factors involved still wait to be identified.
In order to point up HIF-1 independent mechanisms triggered under hypoxia, we decided to use, for comparative analysis, the murine cell line Hepa C1 and its counterpart Hepa C4, which lacks the ARNT subunit. A proteomic 2-D gel using the Cye Dye (DIGE) technique allowed us, after stimulation (normoxia versus hypoxia) of 3 independent cell cultures, to highlight different proteins that are responsive to hypoxia. These proteins were sorted into two groups: proteins that are overexpressed under hypoxia in both cell lines, and cell line-specific proteins. For the first group, the most striking observation is that all these proteins are up-regulated under hypoxia, there is no down-regulated proteins. Moreover, the fold of induction in Hepa C4 cell line is often bigger than in the Hepa C1 counterpart. This could be interpreted as a part of a compensatory mechanism. Several such proteins were identified by mass spectrometry. They correspond, for example, to Signal Transducing Adaptator protein 2, Ribonucleotide Reductase M1 or Elongation Factor B. For the second group, we focused on proteins overexpressed under hypoxia in Hepa C4, whose induction is HIF-1 independent. They are mostly cytoskeleton proteins like lamin A/C, vimentin, cortactin or dynein, and they are up-regulated or biochemically modified during hypoxia in Hepa C4 cells. We also pointed out the up-regulation of the chaperonine TCP-1 epsilon, which is known to interact with cytoskeleton elements. Their potential involvement into the adaptive responses to hypoxia are currently under investigation, but they may play a role in cell migration that could be enhanced under hypoxia.
All these results evidenced several new HIF-1-independent proteins which are involved in the adaptive response to hypoxia. These proteins may represent new attractive targets for cancer therapy.

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Is Urine a feasable and reliable Tool to screen for HPV?

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Abstract
Aim: Human Papillomavirus (HPV)-infection is the most common sexual transmitted disease (STD) of viral aetiology. At least 30 types of HPV are known to infect the ano-genital region. Low-risk types (6, 11) are responsible for rather benign conditions like genital warts while high-risk types (16, 18, 31, 33, 45) are related with ano-genital cancer like cervical and penile carcinomas. Cervical cytology can detect (pre)malignant lesions in asymptomatic women. In abnormal smears it is recommended to perform Polymerase Chain Reaction (PCR)-testing for HPV-DNA, which is the golden standard. It can be used for screening, diagnostic and follow-up purposes. For males there is no golden standard at all. For both sexes a cheap and reliable method should be found; as well for diagnostics, as for screening and follow up. Testing urine for HPV could be a possibility since screening kits for other STD's are available using PCR-testing of first voided urine specimens (FVU). To evaluate if HPV testing on urine is a reliable technique, ß-globin concentration and HPV positivity for oncogenic HPV types was measured by real time PCR in FVU and compared with the findings in swabs of the glandular urethra and of the glans penis. The concentration of ß-globin and HPV positivity in FVU of males and females was also compared.
Methods: First voided urine (FVU) of 30 males and 31 females and urethra-glandular brushes of 12 men were examined for the presence of ß-globin (indicative for the amount of DNA). Then HPV-DNA was searched using PCR.
Results: The percentage of FVU-samples with detectable ß-globin concentration did not differ between men (66.7%) and women (71%). Where the Cervex brush was used to collect glandular-urethral cells, ß-globin was detected in 100% of the cases. Correspondingly the mean absolute concentration of DNA (ng/µl) is significantly higher in the brushes: 0.9985 ng/µl versus 0.0207 ng/µl (FVU men) and 0.0916 ng/µl (FVU females). This results in HPV-positivity in 66.7% of the brushes versus 0% of FVU in males and 4.5% of FVU in females.
Conclusion: FVU in men (male and female) is not a good tool, nor for diagnostics, nor for screening. While the presence of ß-globin is moderately high, the absolute amount of DNA is to low to detect the presence of HPV.
ß-globin-positivity of urethral swabs is 100%, showing an higher absolute concentration of DNA, leading to a higher detection rate of HPV.

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NF-kappaB signature of inflammatory breast cancer by cDNA microarray validated by quantitative real-time RT-PCR and immunohistochemistry.

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Inflammatory Breast Cancer (IBC) is the most aggressive form of locally advanced breast cancer with high metastatic potential. Most patients at diagnosis have lymph node involvement and 1/3 of the patients have metastasis in distant organs. In a previous study we demonstrated that IBC is a different form of breast cancer compared to non-IBC by cDNA microarray analysis. A list of 756 genes with significant gene expression differences between IBC and non-IBC was identified. Unsupervised hierarchical clustering separated IBC from non-IBC. A set of 50 discriminator genes was identified in a learning group of tumour samples and was successful in diagnosing IBC in a validation group of samples (accuracy of 88%). In depth functional analysis revealed the presence of a high number of NF-kappaB target genes with elevated expression in IBC versus non-IBC. This led to the hypothesis that NF-kappaB is an important factor, contributing to the development of IBC. The aim of the present study was to further investigate the role of NF-kappaB in the pathogenesis of IBC. Therefore, we analyzed the expression of 8 NF-kappaB target genes with significant differences (at least 3 fold) in gene expression levels between 9 IBC and 9 non-IBC specimen, measured by cDNA microarray analysis (Van Laere et al, Breast Cancer Res Treat, in press). Additionally, we stained 44 IBC and 45 non-IBC tissue sections using antibodies against the NF-kappaB family member RelA, RelB, cRel, NFkB1 and NFkB2. We found a significant overexpression for all NF-kappaB target genes in IBC compared to non-IBC. Furthermore, we found strong correlations between all NF-kappaB target genes, indicative for a common transcriptional regulation. In addition we found a statistically elevated number of stained nuclei in IBC as compared to non-IBC for RelB (p=0.038) and NFkB1 (p<0.001). Transcriptionally active NF-kappaB dimers, composed of specific combinations of NF-kappaB family members, were found in 17/44 IBC specimens compared to 2/45 non-IBC specimens using a 50% cut-off value for discrimination between absence and presence for each NF-kappaB family member (p<0,001). The same analysis was repeated using cut-off values of 40% and 30%. We identified respectively 23/44 and 32/44 IBC specimen with transcriptionally active NF-kappaB dimers compared to respectively 7/45 and 16/45 non-IBC specimen with transcriptionally active NF-kappaB dimers (Pearson χ^2, p<0.001 and p<0.001 respectively). In order to validate these results we looked for gene expression differences for NF-kappaB target genes between tumours with transcriptionally active and inactive NF-kappaB dimers. Independent of the chosen cut-off value, we found significant gene expression differences for most of the NF-kappaB target genes with higher median expression of NF-kappaB target genes in tumours with transcriptionally active NF-kappaB dimers. These results clearly demonstrate that NF-kappaB, and particularly the RelB/NFkB1 dimer, is activated more often in IBC compared to non-IBC. In conclusion, the NF-kappaB transcription factor pathway probably contributes to the phenotype of IBC. We are currently performing NF-kappaB DNA-binding experiments to further validate the NF-kappaB signature of IBC.
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Identification of cell-of-origin breast tumour subtypes in Inflammatory Breast Cancer by gene expression profiling.

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Inflammatory Breast Cancer (IBC) is an aggressive form of locally advanced breast cancer with high metastatic potential. Most patients have lymph node involvement at the time of diagnosis and 1/3 of the patients have distant metastases. In a previous study, we demonstrated that IBC is a distinct form of breast cancer in comparison with non-IBC (Van Laere et al, Breast Cancer Res Treat, 2005). The aim of this study was to investigate the presence of the different molecular subtypes, described by Sorlie et al (PNAS,2003) in our data set of 16 IBC and 18 non-IBC specimen. Therefore, we selected an "intrinsic gene set" of 144 genes, present on our cDNA chips and common to the "intrinsic gene set" defined by Sorlie et al (PNAS, 2003). This set of genes was tested for performance in the Norway/Stanford data set by unsupervised hierarchical clustering. 84% Of the samples belonging to this set clustered in the same manner as described in the original manuscript. Expression centroids were then calculated for the core members of each of the 5 subclasses in the Norway/Stanford data set and used to classify our own specimens by calculating spearman correlations between each sample and each centroid. We found a higher amount of basal-like and ErbB2-overexpressing tumours and a lower amount of luminal A, luminal B or normal-like tumours in IBC as compared to non-IBC (ErbB2+/Basal: IBC:8, non-IBC:3; LumA/LumB/Norm: IBC:8, non-IBC:15; Pearson Chi-Square, p=0.036). Our findings were in good agreement with protein expression data for Estrogen Receptor (ER+; ErbB2+/Basal: 2/11, LumA/LumB/Norm: 12/23, p=0.063) and Cytokeratin 5/6, a representative marker for the basal-like subtype (CK5/6+; basal: 6/7, non-basal: 4/13, p=0.057). The classification was confirmed by using an alternative unsupervised hierarchical clustering method (average-linkage clustering). The robustness of this classification was confirmed by unsupervised hierarchical clustering using an alternative gene set of 141 genes related to the cell-of-origin subtypes, selected using a discriminating score and 200 iterative random permutations producing less then 5 false positives. Compared to the data published by Bertucci et al (Cancer Research, 2005), we found a higher amount of basal and Her2 overexpressing tumours and a lower amount of luminal A, luminal B or normal-like tumours in IBC as compared to non-IBC (Her2/Basal: IBC:8, non-IBC:3; LumA/LumB/Norm: IBC:8, non-IBC:15; Pearson Chi-Square, p=0.036). Furthermore, we found a significant difference in the amount of ER+ IBC specimen in the combined Luminal A, Luminal B and Normal-like clusters as compared to the corresponding cluster reported by Bertucci et al (respectively 4/8, 19/19, Fisher's Exact Test; p=0.004). The presence of the same molecular cell-of-origin subtypes in IBC as in non-IBC does not exclude the specific molecular nature of IBC. The molecular mechanisms involved in the biology of IBC are translated in gene sets, different from the ones used to define cell-of-origin subtypes. Therefore we suggest that IBC and non-IBC have to be regarded as separate entities with important diagnostic and therapeutic consequences.
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Inflammatory Breast Cancer: At the cross-roads of NF-kappaB and Estrogen Receptor signaling pathways.

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Inflammatory Breast Cancer (IBC) is the most aggressive form of locally advanced breast cancer characterized by a high metastatic potential and poor patient outcome. In a previous study we demonstrated that IBC is a different form of breast cancer compared to non-IBC by cDNA microarray analysis. A list of 756 genes with significant gene expression differences between IBC and non-IBC was identified. Among these genes a large amount of NF-kappaB target genes was detected. The NF-kappaB signature in IBC was validated by Real-Time RT-PCR for 8 NF-kappaB target genes and by immunohistochemistry for all NF-kappaB transcription factor family members (RelA, RelB, cRel, NFkB1 and NFkB2). Recently, several studies have indicated a cross-talk between Estrogen Receptor (ER) and NF-kappaB signaling, resulting in the inhibition of NF-kappaB or ER dependent signaling. Since IBC is known to be more often ER negative compared to non-IBC, we hypothesize that the presence of the NF-kappaB signature in IBC can be explained by the interaction between both signaling pathways. To investigate this hypothesis we performed Real-Time RT-PCR on 17 IBC and 20 non-IBC specimens for ER alpha, ER beta and Progesterone Receptor (PR). The expression of NF-kappaB target genes, measured by Real-Time RT-PCR was correlated with gene and protein expression data for ER. Immunohistochemical data for transcriptionally active NF-kappaB dimers were correlated with IHC and gene expression data for ER and PR. As expected, relative gene expression of ER alpha en PR were elevated in non-IBC (median respectively 5,93 and 1,17) compared to IBC (median respectively 0,75 and 0,71) specimens, however only the difference in expression for ER alpha showed a trend towards significance (p=0,069 and p=0,68). Relative gene expression levels for ER beta was significantly elevated in IBC (median 1,46) compared to non-IBC (median 0,78) (p=0,013). The expression of all NF-kappaB target genes were significantly elevated in IHC ER- tumours compared to IHC ER+ tumours. The expression levels of all NF-kappaB target genes showed a significant inverse correlation with the expression level of ER alpha, whereas for PR inverse correlations were observed for only 3 NF-kappaB target genes. For ER beta gene expression, significant correlations were found with 5 NF-kappaB target genes. When analyzing the presence of transcriptionally active NF-kappaB dimers in IHC ER+ *vs* IHC ER- tumour specimens we found 16/49 ER- tumours and 3/40 ER+ tumours with transcriptionally active NF-kappaB (p<0,004). The median gene expression level for both ER alpha and PR is reduced in IHC NF-kappaB positive tumours (median respectively 0,68 and 0,50) compared to IHC NF-kappaB negative tumours (median respectively 4,41 and 1,31) (p=0,179 and p=0,304). The median relative gene expression level for ER beta is elevated in IHC NF-kappaB positive tumours (median 2,46) compared to IHC NF-kappaB negative tumours (median 0,79) (p=0,122). Altogether, these data suggest a cross-talk between ER signaling and NF-kappaB signaling in IBC with potential therapeutic implications. We are currently investigating an ER target gene signature in our data set of IBC and non-IBC specimens and ER DNA-binding assays will be performed.
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Inflammatory breast cancer has a quiescent fibroblast gene expression signature

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Introduction : Chang et al. demonstrated that breast cancer and other solid tumours can be devided in two groups with different prognosis based on a wound healing gene expression profile, obtained by comparing quiescent and activated fibroblasts (Chang et al. PLos Biology 2004 and PNAS 2005) . We investigated whether this wound healing signature could be found in the specific and distinct gene expression profile of inflammatory breasts cancer (IBC) (Van Laere et al. Breast Cancer Res Treat, in press).
Materials and Methods : A set was compiled of genes being part of the quiescent and activated fibroblasts signature (Chang et al. Plos Biology 2004) that were also present on the cDNA microarrays used to compare IBC and non-IBC specimens (Van Laere et al. Breast Cancer Res Treat, in press). Its performance was tested on Chang's dataset. A group op samples of quiescent and of activated fibroblasts with the most homogenous gene expression were used to calculate the respective centroids. The power was tested. With this reduced gene set, IBC and non-IBC specimens were hierarchically clustered and correlations between gene expression of each IBC and non-IBC specimen and both centroids were calculated. The same analyses were performed after exclusion of proliferation-related genes (Chang et al. Plos Biology 2004))
Results : The compiled gene set consisted of 98 genes. Hierarchical cluster analysis perfectly separated quiescent and activated fibroblast samples, validating its use on our IBC and non-IBC dataset. 15 and 18 samples were used to calculate the quiescent and activated centroids in Chang's dataset. All remaining samples of quiescent and activated fibroblasts correlated to the quiescent and activated centroid, respectively: power 100%. Table 1 shows the results of the correlation analysis of IBC and non-IBC specimens with the centroids. When proliferation-related genes were excluded, 78 out of 98 genes were left. With this gene list, similar results were found. The differential expression of wound-healing genes in IBC and non-IBC is momentarily confirmed using real-time RT-PCR.
Discussion : Our results show that the gene signature of Chang et al. can differentiate between IBC and non-IBC and that IBC mostly shows a quiescent gene expression signature. These results again confirm that IBC is a distinct biological entity with a specific gene expression signature. The quiescent expression phenotype could be correlated to the infiltrative growth pattern of IBC. In IBC, tumour cells indeed grow between pre-existing breast structures without destruction of the stroma or induction of a desmoplastic reaction.

Table 1

p = 0.006 (Sign + Non-Sign); p = 0.03 (Sign Only)		IBC		Non-IBC	
Quiescent Centroid	Significant	6	**12**	3	**5**
	Non-Significant	6		2	
Activated Centroid	Significant	0	**4**	5	**13**
	Non-Significant	4		8	

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Real-time RT-PCR of CD146 and VE-cadherin mRNA to detect circulating endothelial cells in peripheral blood of patients with breast cancer

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Introduction: Angiogenesis is a fundamental process in tumour growth and metastatic dissemination. The number of circulating endothelial cells (CECs) in peripheral blood (PB) of patients with cancer reflects the amount of proceeding neoangiogenesis and can therefore be used as a surrogate marker to monitor antiangiogenic therapy. The standard quantification method of CECs is currently based on a complex four-color flow cytometrical analysis. However, real-time RT-PCR technology to quantify endothelial cell-specific mRNA in PB samples has been shown to be a promising alternative approach. This study aimed to compare mRNA expression levels of endothelial-cell specific markers (CD146 and VE-cadherin) in PB of healthy volunteers and patients with breast cancer using real-time RT-PCR.
Methods: PB samples have been collected from 18 healthy volunteers and 18 metastatic breast cancer patients using the PAXgene Blood RNA System. RNA was subsequently isolated with the PAXgene Blood RNA isolation kit according to manufacturer's instructions and reverse transcribed into cDNA with random primers. Real-time PCR analysis was performed with primers and TaqMan probes for both CD146 and VE-cadherin mRNA. Ct values were normalised for beta-actin mRNA expression and gene expression levels were calculated relative to a reference sample (RGE).
Results: VE-cadherin mRNA was increased in patients with breast cancer in comparison to healthy volunteers: the median VE-cadherin mRNA expression level in PB of healthy volunteers was 1.20 (range 0.50-4.18); while this was 2.45 (range 0.69-25.80) for patients with breast cancer (p=0.040). However, the difference in CD146 mRNA expression levels between healthy volunteers and patients with breast cancer did not reach statistical significance: the median CD146 mRNA expression level in PB of healthy volunteers was 0.037 (range 0.020-0.058); while this was 0.058 (range 0.013-0.488) for patients with breast cancer (p=0.077). CD146 and VE-cadherin mRNA expression levels were significantly correlated (r=0.401, p=0.017). A cut-off value was determined as the 95th percentile of the RGE values of the healthy volunteers: this value was 0.058 for CD146 and 4.184 for VE-cadherin mRNA. 9 out of 17 patients with breast cancer had a RGE of CD146 above the cut-off value; while for VE-cadherin 7 out of 18 patients with breast cancer had increased RGEs.
Discussion: Our preliminary results suggest that the quantitative evaluation of endothelial cell-specific mRNA by real-time RT-PCR technology could indeed be a promising tool to monitor the efficiency of antiangiogenic therapy in patients with breast cancer but a larger study population and a comparison with flow cytometry is necessary to confirm this. These studies are ongoing.

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The Differential Expression of Galectin-1 and Galectin-3 in Normal Lymphoid Tissue and Non-Hodgkin's and Hodgkin's Lymphomas.

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ABSTRACT :
The WHO classification of lymphomas was established on the basis of clinical, morphological, immunohistochemical and genetic criteria. However, each entity displays its own spectrum of clinical aggressiveness. Treatment success varies widely and is not predictable. Since galectins are involved in oncogenesis and the physiology of immune cells, we investigated whether galectin-1 and galectin-3 immunohistochemical expression could differ in 25 normal lymphoid tissues, 42 non-Hodgkin's and 14 Hodgkin's lymphomas. Immunohistochemical galectin expression was submitted to semi-quantitative and quantitative (computer-assisted microscopy) evaluations. This study is completed by an analysis (by means of quantitative RT-PCR) of galectin-3 mRNA expression in 3 normal lymph nodes, 3 follicular lymphomas (FLs) and 3 diffuse large B-cell lymphomas (DLBCLs). The data show that in normal lymphoid tissue, lymphocytes do not express galectin-1 and rarely express galectin-3. In contrast, galectin-3 was expressed in 8 of the 16 DLBCL cases and in 1 of the 8 FL cases. Furthermore, galectin-3 mRNA was expressed 3-times more in the DLBCLs than in the FLs. While the blood vessel walls of the lymphomas expressed galectin-1, the vessel walls of normal lymphoid tissues did not. This expression of galectin-1 in blood vessel walls was correlated with vascular density. The present study thus shows that DLBCL can be distinguished from normal lymphoid tissue and other lymphomas on the basis of galectin-3 expression. In addition, galectin-1 might be involved in lymphoma angiogenesis.
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Role of PKA in HIF-1α phosphorylation in response to intermittent hypoxia.

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It is now well established that vascularized tumors are exposed to intermittent hypoxia, that is, hypoxia followed by periods of reoxygenation. Abnormal structure and dysfunction of tumor blood vessels are responsible for these conditions. These repeated short periods of hypoxia concern tumor cells as well as endothelial cells. However, the effects of intermittent hypoxia on endothelial cells are poorly understood. The aim of this study is to investigate the effects of intermittent hypoxia on endothelial cells and particularly on HIF-1α, a central actor in adaptive response to hypoxia.
In order to study the effect of intermittent hypoxia, EAhy926 endothelial cells were exposed to repeated cycles of hypoxia/reoxygenation. Four repeated cycles of one-hour hypoxia followed by thirty minutes reoxygenation were used for a total period of 6 hours.
A modification of HIF-1α phosphorylation pattern under intermittent hypoxia was evidenced. Indeed, in the course of hypoxia/reoxygenation cycles, a gradual increase in the abundance of the HIF-1α phosphorylated form with respect to its non-phosphorylated form was observed.
The possible involvement of different kinases, which could phosphorylate HIF-1α under hypoxia, was then investigated. The phosphorylation of p42/p44 MAPKs and AKT was followed. An increase in their phosphorylation in the course of hypoxia/reoxygebation cycles was observed and a maximal phosphorylation of p42, p44 and AKT was noticed when the abundance of the HIF-1α phosphorylated form was maximal. Treatment with PD98059 (a MAPKK inhibitor) or LY294002 (a PI3K inhibitor) induced a decrease in respectively MAPKs and AKT phosphorylation. However, no modification in HIF-1α phosphorylation pattern was observed in the presence of these inhibitors, indicating that these kinases are problably not involved.
Other candidates were then investigated: different inhibitors of kinases were used and a modification in HIF-1α phosphorylation pattern was observed in the presence of a PKA inhibitor (H-89). An increase in the phosphorylation of CREB (a well known PKA substrate) was observed with a maximal phosphorylation when the abundance of the HIF-1α phosphorylated form was maximal. Incubation in the presence of H-89 induced a decrease in the abundance of phopho-CREB but also in the abundance of the HIF-1α phosphorylated form.
In conclusion, we showed that repeated cycles of hypoxia/reoxygenation induced a modification in HIF-1α-phosphorylation pattern in EAhy926 endothelial cells. Activation of p42/p44 MAPKs, AKT and PKA was observed in these conditions. The use of inhibitors indicates that PKA seems to be involved in the phosphorylation of HIF-1α under intermittent hypoxia, while p42/p44 MAPKs and AKT are not. A better knowledge of the effects of intermittent hypoxia on endothelial cells and the highlight of particular mechanisms induced by intermittent hypoxia are essential to understand the behaviour of endothelial cells during neo-angiogenesis.

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Specific enzyme-mediated anticancer drug delivery to melanoma cells

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Most of the conventional anticancer drugs currently in use are based on a differential uptake by tumor cells due to their higher metabolism/proliferation. Unfortunately, they also affect normal tissues and produce general toxic side effects. Several approaches are being explored to improve drug delivery using different systems. This study addresses one of these mechanisms by using tumor associated proteases as targets for specific drug delivery. We found that some alkylating peptides belonging to the old drug Peptichemio are processed efficiently by tumor proteases. Our present work focuses on one of these: PSF (L-prolyl-m-L-sarcolysyl-L-p-fluorophenylalanine-ethylester) in terms of stability, transport, metabolisation and cytotoxic effect.
While PSF was stable in human plasma, it disappeared very quickly in whole blood along with the generation of its main metabolite. The presence of Red Blood Cells (RBC) membranes was required for both binding and generation of the metabolites. When RBC ghosts were incubated with PSF, a higher generation of m-sarcolysin compared to the incubation of PSF with whole RBC was observed. PSF was able to bind the RBC ghosts membranes and could be processed by the associated proteolytic enzymes.
Enzymatic catalysis was further supported by a set of experiments where the enzymatic activity was inhibited in various ways (e.g. use of protease inhibitors). PSF was exposed to 3 extracellular matrix proteases: Collagenase A had no effect, but both Dispase and Trypsine were able to process PSF. Melanoma Cells (MC) showed a higher capacity than RBC to bind and process PSF both by membrane-associated and soluble proteases. PSF showed the same cytotoxicity profiles towards MC with 2 or 48h exposure indicating a rapid uptake. MC were able to completely and rapidly generate the active metabolites from RBC-bound PSF. Body distribution of ^{14}C-labeled PSF in human melanoma bearing nude mice showed a significant increase of accumulation only in the tumor tissue. A dose finding study in nude mice allowed us to administer up to 20 mg/kg IP single dose that was able to induce, unlike an equivalent dose of melphalan or m-SL, not only inhibition of growth but also tumor regression without any significant weight loss. Dose-response curves where achieved and fractionated doses had significant better effects on tumor regression and regrowth than a single dose.
Our results strongly suggest a particular delivery system based on a rapid binding to BC, subsequent transport, followed by a proteolytic activation at the targeted tumor sites, drug targeting being achieved by the much higher load in proteolytic enzymes in tumors. Drug delivery is accomplished through an active competition between BC-membrane bound enzymes and, soluble as well as tumor-membrane-associated peptidases.

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The radiosensitizing effect of gemcitabine and apoptosis: Extrinsic or intrinsic activation?

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Both in clinical and preclinical studies it is shown that gemcitabine has clear radiosensitizing properties. This radiosensitizing effect is dependent of the cell cycle effect, an early S phase block followed by a G_2/M block, and an increased induction of apoptosis. This study was performed to clarify the apoptotic pathway responsible for radiosensitization by gemcitabine. We investigated the activation of caspase 8 and 9, both initiator caspases of the extrinsic or intrinsic pathway, respectively.
The cell lines used for this study were ECV304 (mt-p53), a human bladder cancer cell line and H292 (wt-p53), a human mucoepidermoid lung cancer cell line. The cells were treated with gemcitabine (IC25 and IC90) during 24 h, radiotherapy (γ-Co^{60}, 0-6 Gy, room temperature), or with the combination, i.e. gemcitabine immediately followed by radiation. 72h after treatment, apoptotic cell death is determined using TUNEL assay, Annexin V staining and caspase 3 activity assay. Caspase 8 and 9 activation is determined by Western blot at different time points (0-72h) after gemcitabine or radiotherapy and 72 h after the combination. Using the mitochondria-selective probe (tetramethylrhodamine methylester) and flow cytometry, the mitochondrial membrane potential (ψ) was determined.
72 h after treatment, the combination of gemcitabine and radiotherapy resulted in a clear increase of apoptosis in both cell lines. At that moment, both procaspase 8 and 9 cleaving was observed with Western blot. In addition, ψ was reduced when the amount of annexin V positive cells increases.
To determine the order of caspase 8 and 9 activation, caspase cleaving was determined at different time points after gemcitabine or radiotherapy. In both settings, caspase 8 activation was observed at the different time points after treatment, while no cleaving products of caspase 9 could be shown.
In conclusion, treatment of ECV304 and H292 cells under radiosensitizing conditions resulted in an increased induction of apoptosis with caspase 8 and 9 cleaving and a reduction of ψ. This means that both the extrinsic and the intrinsic pathway is activated. However, treatment with gemcitabine or radiotherapy alone resulted only in activation of the extrinsic pathway. Further research is necessary to investigate the caspase 9 activation under radiosensitizing conditions. This could be a result of the increased apoptosis by the combination of gemcitabine and radiation or by Bid activation, the link between the intrinsic and the extrinsic pathway.

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Combined Pap and HPV testing in primary screening for cervical abnormalities: should HPV detection be delayed until age 35?

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Background: In 2003, the United States Food and Drug Administration has approved the Hybrid Capture 2 assay for use with a Pap test to adjunctively screen women of 30 years and older for the presence of high-risk HPV infection. Although the predictive power of a negative test is strong, the number of false-positives may still be high. We investigated HPV prevalence in relation to age in a group of 2,293 women, aged between 20 and 50, with normal cytology.
Methods: Residual material from thin-layer cytology was used for DNA isolation. DNA integrity was checked by beta-globin PCR and HPV DNA was detected by the GP5+/6+ general primer PCR, followed by non-radioactive enzyme immunoassay detection. Probes for 14 high-risk types were available.
Results and discussion: Overall HR-HPV prevalence was 6.9% (95%CI = 5.9 – 8.0%). The age-related HR-HPV prevalence was analysed in 10-year intervals, in 5-year intervals and in intervals of 1 year. Using 1-year intervals, peaks were found at the ages 22, 23 (11.6 and 12.5%, respectively) and at the ages 31, 32 (12.4 and 11.6%, respectively). Using 5-year intervals the HPV prevalence remained stable at approximately 9% in the age groups 20-24, 25-29 and 30-34, and decreased significantly to approximately 5% in the age groups 35-39, 40-44 and 45-50. This would suggest that postponing HPV detection in primary screening from age 30 to age 35 would result in a decrease of almost 50% of the number of women with normal cytology and a transient HPV infection, potentially saving thousands of women in North-Western Europe from being diagnosed with a wide-spread sexually transmitted infection. Investigation of the emotional and psychosocial impact of being diagnosed with HPV showed that women commonly experienced emotional distress (anger, anxiety, depression, fear of rejection, shame and guilt), sexual problems and a negative self-image. Careful consideration has to be taken whether the positive side of HPV detection (detection of women with high-grade lesions, missed by cytology) outweighs the negative side (distress in HPV positive women without cervical lesions). Our data suggest that these opposites are more balanced at a higher age.

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Schedule dependency of the radiosensitising effect of vinflunine and the role of its cell cycle effects, in vitro.

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Vinflunine proved to have radiosensitising potential and caused a clear G2/M blockade (Simoens et al., CCP, in press). However, further investigation was necessary to elucidate the exact role of this cell cycle effect. The purpose of this study was to correlate the cell cycle effects of vinflunine with the radiosensitising effect using different treatment schedules.
ECV304, a human bladder cancer, and CAL-27, a human head and neck cancer cell line were treated with radiosensitising vinflunine-concentrations (50nM and 20nM, respectively). To investigate the influence of the incubation time, cells were treated during 8, 24 or 48hrs prior to radiation (Co-60γ rays, 0-8 Gy, room temperature). The influence of a time interval was tested by 24h incubation with vinflunine, 0, 8 or 24hrs before irradiation (24+0, 24+8, 24+24). Cell survival was determined by the sulforhodamine B assay 7 or 8 days after radiation and radiosensitisation was represented by the Dose Enhancement Factor (DEF). To determine the role of the cell cycle perturbations caused by vinflunine at the moment of radiation, cells were analysed by flow cytometry (DNA staining). Cells were treated with 150nM (ECV304) and 100nM vinflunine (CAL-27) using the above mentioned treatment schedules.
In both ECV304 and CAL-27, 8h of incubation did not resulted in a radiosensitising effect, although a significant increase in G2/M phase cells was observed. This increase was probably not strong enough to compensate for the simultaneous significant increase in S phase cells (most radioresistant) at that time. Maximum radiosensitisation was reached after 24h incubation (maximum G2/M block) and decreased with longer incubation times (48h), due to a decrease of G2/M phase cells and an increased polyploid population.
Using different time intervals, maximum radiosensitisation (*DEF=2,01*) was observed after 24h incubation immediately followed by radiation (24+0) in ECV304. This also resulted in the largest arrest at G2/M (*57,3% versus 17,8% in control cells*). Radiosensitisation gradually decreased with an increasing time interval between vinflunine-treatment and radiation, due to a decrease in G2/M, an increase in G1 (24+8) and an increase in the polyploid population (24+24). In CAL-27, radiosensitisation was most pronounced after an 8h interval between vinflunine-treatment and radiation (24+8) (*DEF=2,36 versus 1,79 immediately after the 24h incubation period (24+0)*). The only linked cell cycle effect was the lowest amount of S phase cells at that time, although the amount of G2/M cells was already significantly decreased compared to 24+0. A 24h interval (24+24) resulted in a strong decrease of radiosensitisation, together with the highest amount of cells in S and the polyploid population.
In conclusion, the radiosensitising effect of vinflunine after different treatment schedules is cell line-dependent. The observed G2/M block cannot explain the altered radiosensitivity all by itself. Other cell cycle effects, like the presence of S phase and polyploid cells, also play an important role in the radiosensitivity after vinflunine-treatment. Possibly, other non-cell cycle-related causes may exist.

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In vitro cytotoxic activity of pemetrexed alone and in combination with irradiation.

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Introduction: Pemetrexed (Alimta, LY231514, Eli Lilly and Co.) is a novel generation antifolate that targets multiple folate-dependent enzymes involved in purine and pyrimidine synthesis. It has been shown that pemetrexed exhibits antitumour activity against a broad spectrum of human malignancies, including pancreatic, lung, mesothelioma, head and neck and cervical cancers. Since these tumour types are often treated using chemotherapy in combination with radiotherapy, pemetrexed might be an interesting cytostatic agent to incorporate in chemoradiation protocols. Indeed, previous *in vitro* studies suggest a moderate radiosensitising effect of pemetrexed.
This study focuses on the *in vitro* cytotoxic activity of pemetrexed in 3 human tumour cell lines. Moreover, the interaction between pemetrexed and irradiation is investigated using different treatment schedules.
Materials & methods: The human tumour cell lines examined are CAL-27 (a squamous cell carcinoma cell line of the tongue), A549 (a lung carcinoma cell line) and PANC-1 (an epitheloid carcinoma cell line of the pancreas). For the cytotoxicity experiments, cells were incubated with pemetrexed (0 – 1500 µM) for 24h. In combination with radiotherapy, cells were treated with pemetrexed for 24 h immediately before or after radiation (γ-Co^{60}, 0 – 8 Gy, room temperature). Cell survival was determined by the sulforhodamine B assay. For the cytotoxicity assays, the concentration pemetrexed resulting in 50 % growth inhibition (IC_{50}) was calculated. Dose survival curves for the combination studies with radiation were fitted according to the linear-quadratic model and the ID_{50} represented the radiation dose producing 50 % growth inhibition. The potential radiosensitising effect was assessed by calculation of the dose enhancement factor (DEF, ID_{50} control/ID_{50} combination).
Results: For CAL-27 and A549 cells, the IC_{50} for pemetrexed was situated in the nanomolar range (123.7 ± 19.3 nM and 639.9 ± 68.8 nM respectively). In PANC-1 cells however, the cytotoxic effect of pemetrexed was much less pronounced and concentrations as high as 1500 µM resulted in only 30 % growth inhibition.
In combination with radiotherapy, pemetrexed exposure (concentrations ≤ IC_{60}) 24 h before irradiation was unable to mediate a clear radiosensitising effect in CAL-27 and A549 cells. For both cell lines, the DEF was about 1.00 for different concentrations pemetrexed, indicating only additive toxicities. In CAL-27 cells, the inverse schedule (i.e. irradiation followed by treatment with pemetrexed) could not establish radiosensitisation either.
Conclusion: The *in vitro* cytotoxic activity of the novel folate antimetabolite pemetrexed is clearly concentration and cell line dependent. In combination with radiation, no radiosensitising effect could be demonstrated so far, but further experiments are necessary.

In vitro interaction between pemetrexed and gemcitabine using different treatment schedules.

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Introduction: Pemetrexed (Alimta, LY231514, Eli Lilly and Co.) is a novel antimetabolite that targets multiple folate-dependent enzymes involved in purine and pyrimidine synthesis. Pemetrexed and gemcitabine (a deoxycytidine analogue) both have shown preclinical and clinical activity against a broad spectrum of tumour types. Based on their different mechanisms of action and their non-overlapping toxic side effects, the combination of these two antimetabolites seems promising. The present study investigates the interaction between pemetrexed and gemcitabine using different treatment schedules in 2 human tumour cell lines.
Materials & methods: The human tumour cell lines examined are CAL-27 (a squamous cell carcinoma cell line of the tongue) and A549 (a lung carcinoma cell line). Cells were treated with pemetrexed alone (0 – 2000 nM), gemcitabine alone (0 – 100 nM) or with a combination of both chemotherapeutics where one drug was added at a fixed concentration, while the other drug was added in a concentration range. Three different combination regimens were tested: (1) simultaneous exposure to pemetrexed and gemcitabine for 24 h; (2) gemcitabine treatment for 24 h immediately followed by pemetrexed for 24 h and (3) the reverse sequence, i.e. pemetrexed for 24 h immediately followed by exposure to gemcitabine for 24 h. Cell survival was determined by the sulforhodamine B assay and drug interaction was evaluated by median drug analysis by calculating the combination index (CI).
Results: A simultaneous treatment using a concentration range of pemetrexed and a fixed, non-toxic concentration of gemcitabine or vice versa, resulted in CAL-27 cells in an additive to synergistic effect with low concentrations and in an antagonistic effect using higher concentrations. Both schedules resulted in antagonism in A549 cells.
When the cells were treated during 24 h with pemetrexed immediately followed by a 24 h incubation with gemcitabine, using a concentration range or a fixed dose, an additive to synergistic effect is observed in CAL-27 cells in the lower concentration range, while moderate antagonism is observed at higher concentrations. In A549, this schedule resulted only in an antagonistic to additive effect. When 24 h gemcitabine treatment is followed by pemetrexed for 24 h, the same trend is observed in CAL-27 and A549 cells as the previous schedule.
Conclusion: So far, it is difficult to determine the optimal combination regimen of pemetrexed and gemcitabine. Therefore, a third cell line and additional treatment schedules will be investigated. At present, our experiments indicate that the *in vitro* interaction between pemetrexed and gemcitabine is clearly dependent on the cell line, the concentration of the drugs and the treatment regimen used.

Prognostic significance of microsatellite instability in a Belgian colorectal cancer study population.

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The development of colorectal cancer (CRC) is heterogeneous involving several genetic pathways, including chromosomal instability and microsatellite instability (MSI).
In accordance with the strong need for more targeted adjuvant therapy in early stage CRC patients, discriminating prognostic markers for outcome are required.
Although no consensus has been reached, MSI appears to be a promising marker since growing evidence shows differences in outcome, survival and prognosis in MSI- and microsatellite stable (MSS) patients.
To determine the use of MSI as prognostic marker, several clinical and histopathological features as well as the MSI status were evaluated in 199 CRC samples obtained from 189 patients treated at the University Hospital of Antwerp (UA) (123), at Ghent University Hospital (UG) (42), at the Heilig Hart Hospital in Roeselare (24) and at AZ St.-Jan in Bruges (10) to date.
Formalin-fixed, paraffin-embedded blocks were subjected to microdissection in UA while in UG (76), normal tissue was derived from matched peripheral blood leukocytes. The polymerase chain reaction (PCR) was used for MSI-analysis. In addition to the Bethesda reference panel, 5 additional 18S markers were examined in UG. If the Bethesda markers showed a low level of MSI (MSI-L) or less than 5 markers were amplifiable, 5 additional markers were analyzed in UA. The result obtained by UA and UG were in good agreement. A high level of MSI (MSI-H) was defined as having instability in 30-40% or more markers, MSI-L was defined as having instability in less than 30-40% of the markers, whereas in MSS no apparent instability was noted.
Preliminary results indicate that of the samples analyzed to date, 6.5% showed MSI-H, 4% showed MSI-L and 89.5% was MSS. The study comprised 80 females and 109 males. There seems to be a trend towards proximal location (p= 0.06, Fisher's Exact Test) in MSI-H tumors compared to MSI-L/MSS. No major difference is noted in age, sex, tumor stage and differentiation grade (Table).

		MSI-H	%	MSI-L	%	MSS	%
Age		60.1		51.5		56.8	
range		36-73		36-71		29-88	
sex							
	men	7/13	53.8	7/14	50	69/167	41.3
	women	6/13	46.2	7/14	50	98/167	58.7
differentiation							
grade	bad	2/11	18.1	0/12	0.0	8/143	5.6
	bad-medium	1/11	9.1	0/12	0.0	6/143	4.2
	medium	5/11	45.5	8/12	66.7	85/143	59.4
	medium-well	0/11	0.0	1/12	8.3	12/143	8.4
	well	3/11	27.3	3/12	25.0	32/143	22.4
tumor							
location	proximal	8/12	66.7	5/13	38.5	58/166	34.9
	distal	4/12	33.3	8/13	61.5	108/166	65.1
adjuvant							
therapy	no	5/7	71.4	3/11	27.3	20/104	19.2
	chemo	1/7	14.3	6/11	54.5	65/104	62.5
	RT	1/7	14.3	1/11	9.1	2/104	1.9
	chemo + RT	0/7	0.0	1/11	9.1	17/104	16.3

		MSI-H	%	MSI-L	%	MSS	%
TNM							
	T0	0/12	0	0/13	0.0	1/168	0.6
	T1	0/12	0.0	0/13	0.0	5/168	3.0
	T2	2/12	16.7	1/13	7.7	25/168	14.9
	T3	8/12	66.7	6/13	46.2	96/168	57.1
	T4	2/12	16.7	2/13	15.4	22/168	13.1
	Tx	0/12	0.0	4/13	30.8	18/168	10.7
	Tis	0/12	0.0	0/13	0.0	1/168	0.6
	N0	6/12	50.0	4/13	30.8	78/168	46.4
	N1	2/12	16.7	6/13	46.2	50/168	29.8
	N2	3/12	25.0	2/13	15.4	29/168	17.3
	Nx	1/12	8.3	1/13	7.7	11/168	6.5
	M0	4/12	33.3	2/12	16.7	39/169	23.1
	M1	3/12	25.0	5/12	41.7	43/169	25.4
	Mx	5/12	41.7	5/12	41.7	87/169	51.5

Currently full statistical analysis is being performed. Our data and correlations may help to further elucidate the use of MSI analysis as a prognostic factor in sporadic colorectal cancer.

Regulation of the transcriptional activity of human papillomavirus type 16 LCR in choriocarcinoma cell lines.

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Over 100 types of human papillomavirus (HPV) have been identified to date. They all are small circular DNA viruses that infect epithelial cells from various mucosal and cutaneous sites but have strong tissue and species tropism. Because of their association with malignancy, especially cervical cancer, certain HPV types that infect the anogenital mucosa are of particular concern. About 15 types (such as HPV 16 and 18) are termed high risk and are associated with more than 99% of the cervical carcinoma. On the opposite, low risk HPV are only rarely associated with malignancy.
Genital HPV have also been reported in association with spontaneous abortions and development of precancerous (molar pregnancy) or cancerous gestational trophoblastic diseases (choriocarcinoma). Several studies report that HPV could infect trophoblasts during pregnancies. The trophoblast is an embryonic epithelial cell, which plays an essential role in various biological processes such as anchoring, materno-foetal exchanges, immune tolerance and endocrine regulation of the development of the pregnancy. It secretes many hormones such as the hCG (human chorionic gonadotropin) and many cytokines (IL-1, IL-6, IL-8, IL-10, LIF, TNFa, M-CSF, EGF and IFN). Surprisingly HPV can replicate *in vitro* in human trophoblasts.
The early proteins (E) from high risk HPV play a pivotal role in tumor development and have been also reported to disturb some activities involved in the trophoblast differentiation. The transcription of the early region of HPV 16 is initiated at the p97 promoter and is regulated by the long control region (LCR). Both viral (E2) and various cellular transcription factors, including AP-1, C/EBPb, nuclear factor 1 (NF-1), c-myb, Sp1, TEF-1, PEF-1, Oct-1, NF-kB and YY-, have been reported to either activate or inhibit the HPV 16 LCR.
To study the regulation of the HPV16 LCR in four choriocarcinoma cell lines, we transfected all the cell lines with the pWtLCR-Luc plasmid and analysed the luciferase activity expressed under the control of the HPV16 LCR. This activity was relatively high in three cell lines, even higher than in the cervical carcinoma Siha cell line for one of them. Surprisingly, in a fourth choriocarcinoma cell line, the luciferase activity was considerably reduced. It seems that cellular factors secreted by these cell lines could play an important role in the activation or inhibition of the LCR. Although dexamethasone is also a strong activator of the transcriptional activity of the HPV 16 LCR in all choriocarcinoma cell lines, it doesn't seem that endogenous progesterone plays a similar role. The involvement of various transcriptional factors for the HPV16 LCR activity in these trophoblastic cell lines is currently under investigation.

Membrane-Type 4-Matrix Metalloproteinase promotes breast cancer growth and metastases.

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Membrane-type matrix metalloproteinases (MT-MMPs) constitute a subfamily of 6 distinct membrane-associated MMPs. Although the contribution of MT1-MMP during different steps of cancer progression has been well documented, the significance of other MT-MMPs is rather unknown. We have investigated the involvement of MT4-MMP, a glycosylphosphatidyl-inositol (GPI) anchored protease in breast cancer progression. RT-PCR analysis reveals MT4-MMP expression in human breast adenocarcinomas tissues. Interestingly, MT4-MMP protein is strongly detected by immunohistochemistry in epithelial cancer cells of all human breast carcinomas tested and in lymph node metastasis. Stable transfection of MT4-MMP cDNA in human breast adenocarcinoma MDA-MB-231 cells does not affect *in vitro* cell proliferation or invasion, but strongly promotes primary tumor growth and metastatic dissemination in RAG-1 immunodeficient mice. We provide for the first time evidence that MT4-MMP over-production accelerates *in vivo* tumor growth, induces enlargement of blood vessels and increases lung metastasis. These results identify MT4-MMP as a new putative target to design anti-cancer strategies.

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HPV type 16 E7 mRNA electroporated dendritic cells as a possible modality for therapeutic tumor vaccination.

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Human Papillomavirus (HPV) infection is the primary risk factor for cervical cancer, the second most common gynecological cancer among women worldwide. Despite optimized protocols, standard treatments still face several disadvantages. Therefore, novel research aims at the development of immune-based therapies such as loading dendritic cells (DC) with tumor antigens for the induction of anti-tumor immunity.
For this purpose, we – initially - used an HLA-A2-restricted HPV-16 $E7_{11-20}$ peptide for the loading of DC in order to induce a T cell response in vitro. Peptide-pulsed DC were brought into coculture with autologous $CD8^+$ T cells. Following 4 weekly restimulations with peptide-pulsed DC, cultured T cells were subsequently analyzed for antigen specificity by ELISPOT after restimulation with target cells or control cells. With the ELISPOT assay, we detected E7-specific IFN-gamma secretion by $CD8^+$ T cells in 5/5 healthy donors.
There are however some limitations linked to the use of peptides. Peptide-pulsed dendritic cells only express one tumor associated epitope and this in an HLA restricted manner. To overcome these limitations, we explore HPV type 16 E7 mRNA electroporation in dendritic cells in an immune activating setting. We are able to detect the electroporated mRNA by means of RT-PCR several days after electroporation. Moreover, these HPV type 16 E7 mRNA electroporated dendritic cells are capable of stimulating antigen-specific IFN-γ production by a HPV type 16 E7 specific cytotoxic T cell clone. In conclusion, we showed that dendritic cells, antigen-loaded by different means, are able to stimulate a HPV type 16 specific immune response. These experiments concern the development of cultivation protocols for cytotoxic T cells in preclinical vaccination strategies. In future studies we will investigate if mRNA-electroporation will contribute to the generation of a more efficient immune response.

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Think twice before you splice: Constitutive p120ctn exon C expression/depletion affects normal mouse embryogenesis.

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p120 catenin (p120ctn) belongs to the Armadillo family and is the only catenin that is not directly involved in linking cadherins to the actin cytoskeleton. Instead, p120ctn fulfils pleiotropic functions according to its subcellular localisation: (i) modulating the turnover rate of membrane-bound cadherins, (ii) regulating the activation of small Rho GTPases in the cytoplasm and (iii) modulating nuclear transcription. This multifunctionality can be explained by the presence of 48 possible human p120ctn isoforms originating from 4 start codons and 4 alternatively used exons. The alternatively used exon C encodes 6 amino acids that interrupt a nuclear localisation sequence (NLS) in the large insert loop between arm repeats 5 en 6 of the central armadillo domain. This disruption abrogates the inhibition of RhoA. In fact, both the subcellular localisation and the mode of action of p120ctn can be regulated directly by alternative splicing, hence the need for more detailed research on the p120ctn isoforms.
To assess the *in vivo* function of exon C we generated p120ctn exon C-specific knockout (KO-C) and knock-in (KI-C) mice. Offspring derived from crossing heterozygous KO-C mice gave rise only to heterozygous KO-C and wild-type mice in a 2:1 ratio, indicating prenatal mortality. To identify the embryonic stage at which mortality occurs in homozygous KO-C mice, embryos between 7.5 and 16.5 dpc were isolated and genotyped; they too displayed a 2:1 ratio. Paraformaldehyde-fixed paraffin sections of gastrulating embryos (6.0-8.5 dpc) were morphologically staged but no reproducible aberrations could be detected. Remarkably, the constitutive expression of exon C also causes prenatal mortality of homozygous KI-C mice amongst the neonates and 8.5 to 16.5 dpc embryos derived from heterozygous KI-C crosses. Currently we are investigating preimplantation and early-postimplantation stages for KO-C and KI-C mice to provide molecular insight into how disregulated splicing of the smallest exon in the p120ctn gene can have a tremendous effect on the development and viability of an entire organism.

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Nuclear Galectin-3 Expression is an Independent Predictive Factor of Recurrence for Adenocarcinoma and Squamous Cell Carcinoma of the Lung

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Hypothesis/background: The tumor stage is the most powerful prognostic tool for predicting the survival rates of lung carcinoma patients. However, prognosis of individual patients is difficult in part because of the marked clinical heterogeneity among such patients. Galectin-sare involved in cell growth, apoptosis and cell migration features, and their diagnostic and prognostic values have already been demonstrated in various types of cancers. In the present paper we analyze the potential prognostic value of immunohistochemical galectin-3 expression in lung adenocarcinomas and squamous cell carcinomas.
Methods: 165 squamous cell carcinomas and 121 adenocarcinomas were immunostained for galectin-3. In each case the immunohistochemical analyses consisted of an evaluation of the percentage of tumor cells stained and the intensity of staining. An IP score (i.e. Intensity x Percentages) was thus determined for each lung carcinoma.
Results: A large majority of cases displayed galectin-3 expression. While the cytoplasmic staining in the squamous cell carcinomas was focal and moderately intense, the staining in the adenocarcinomas was diffuse and intense. The IP scores were significantly ($p = 0.0001$) higher in the adenocarcinomas than in the squamous cell carcinomas. The difference in nuclear expression profiles between the two cancer types was statistically significant ($p = 0.0005$). Cox multivariate analysis carried out on the patients' genders, the TNM classification and the galectin-3-related variables showed that of the galectin-3-related variables, only the nuclear location of galectin-3 was identified as a prognostic indicator of recurrence independent of the clinicopathological features characterizing the patients ($p = 0.02$). The prognostic contribution of this latter variable was enhanced when the patients with relapse-free follow-ups longer than 8 months were considered ($p = 0.005$).
Conclusions: Galectin-3 immunohistochemical expression differs between squamous cell carcinomas and adenocarcinomas, but the nuclear expression of galectin-3 behaves as a significant prognostic predictor for all the cases as a group.

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Identification of AP-2 cofactors in breast cancers overexpressing ERBB2.

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Introduction
In breast cancer cells, the promoter activity of the oncogene ERBB2 is controlled by transcription factors of AP-2 family, which fix sequences in the promoter region. Like all transcription factors, the AP-2 factors modulate the transcription by interacting with many proteins (cofactors). Contrary to others cellular types, high concentrations of AP-2 factors are transcriptionaly active in breast cancers overexpressing ERBB2. Our assumption is that this characteristic is due to the presence of specific cofactors in breast cancer for AP-2 to remain active. Our goal is to identify these cofactors interacting with AP-2 in breast cancer cells. The techniques undertaken were the affinity chromatography on DNA (ACD) and the technique of GST-pull-down (GPD).
Description of the techniques
These techniques would allow identifying proteins interacting with AP-2. The ACD technique uses specific sequences of DNA including a biotin on 5' end. This biotin allows fixing on streptavidin covering magnetic beads. The GPD uses proteins coupled with the GST (Glutathion Serine Transferase), called chimera proteins (here GST-AP2). This one has an affinity for the glutathion covering magnetic beads. Nuclear proteins extracts from AP-2 overexpressing cells are incubated with these beads then, after washing, the proteins interacting with the DNA or the chimera protein is eluted. After separation of these last on 2 dimensions gel of electrophoresis, the interesting proteins are digested and their peptides separated and analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).
Results
These techniques require many optimizations and have disadvantages differing one from the other. AP-2 α and γ were succefully isolated by the DNA affinity technique and identified by mass spectrometry. The following step was the identification of the interacting proteins. Recently, several proteins were isolated like interacting with AP-2. Their identifications are in progress. When those will be made, the interactions will be confirmed by other techniques and the effect of these proteins on the transcriptional role of AP-2 will be studied by report vectors like by studies of interference with the RNA (siRNA).

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HPV and the ductus deferens

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Abstract
Aim : Human papillomavirus infection (HPV) is causally associated with uro-genital (pre) malignant lesions and carcinomas. It is considered to be a sexual transmitted disease. Although knowledge of HPV infection in women is extensive, limited information is available about the prevalence and behaviour of HPV in men. The role of the male reproductive tract as harbouring reservoir for HPV is poorly understood. The aim of the current study is to investigate the presence of HPV in the vas deferens in a male standard population.
Methods : 30 healthy male patients who had a classic bilateral vasectomy in the University Hospital in Antwerp (UZA) between 1999 and 2004 were included. With the incidence of HPV infection in women in mind, we selected two groups of fifteen men, respectively aged 35 years and younger (group 1) and 45 years and older (group 2). From sections of stored paraffin embedded tissue samples of the vas deferens obtained after the vasectomies, genomic DNA was extracted using the QIAamp DNA mini kit®. HPV DNA detection was performed using real-time polymerase chain reaction (PCR) for 15 oncogenic HPV types (ABI 7500,Applied Biosystems). A separate quantitative real-time ß-globin control PCR was performed to evaluate DNA quality and quantity. We used embedded HELA-cells as a control for DNA extraction and HPV type 18 quantitative real-time PCR. All samples were also tested with the MY 09/11 consensus PCR.
Results : The mean age of the younger group was 32,5 yr (95% CI 10-30), the mean age of the older group 49,9 yr (95% CI 70-100). ß-globin was detected in 90.01% of samples. The mean amount of ß-globin was not significantly different between both groups (younger group 0.8102 ng/µL versus older group 0.7206 ng/µL). All samples were negative for the MY 9/11 consensus PCR. Only 1 sample (3,33%) tested positive for HPV-type 18 in the older group and had a low viral load.
Conclusion : HPV is present in biopsies from vas deferens. The low prevalence could be due to low ß-globin concentration, or because healthy asymptomatic men were included. HPV prevalence is low in asymptomatic healthy men. Extraction of larger amounts of DNA could increase HPV detection.

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Androgens regulation of the ERBB1 and ERBB2 oncogene expression in human prostate cancer cells.

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The only efficient therapy for metastatic prostate cancer consists in androgen ablation that leads to regression of androgen dependent tumours. Nevertheless this response is temporary, and effective therapeutic regimen for tumours that became androgen independent is not yet available. Previous results suggest that tyrosine kinase receptors of the ERBB family might be implicated in prostate cancer progression towards hormone independence by activating the androgen receptor (AR) in androgen-depleted medium. In the present study, we investigated the effect of dihydrotestosterone (DHT) treatment on the ERBB receptors expression in two human prostate cancer cells lines, the LNCaP cells that express the AR and the DU145 cells that do not express the AR.
In LNCaP cells, results of semi-quantitative RT-PCR and western blot analysis show that DHT treatment increases ERBB1 expression and decreases ERBB2 expression both at mRNA and protein levels compared with untreated cells. These effects are time and dose dependent. On the contrary, ERBB3 expression is not changed. No effect is observed in the DU145 cell line. ERBB4 is not expressed in these cells. To see if androgens influence the stability of ERBB1 and ERBB2 mRNA and protein, we estimated the half-life of transcripts and proteins by treating LNCaP cells with respectively actinomycine D or cycloheximide after a DHT pretreatment. mRNA and protein expression were estimated by semi-quantitative RT-PCR and by western-blotting. The half-life of erbB1 and erbB2 mRNA are respectively 6h and 10h with and without DHT. ERBB1 protein half-life is 7h without DHT and increases to 14h with DHT. No effect of DHT treatment was seen for ERBB2 protein that had a half-life of approximately 16h.
These results strongly suggest that androgens act through a transcriptional mechanism to down-regulate ERBB2 transcription. For ERBB1 two mechanisms could be responsible for its increase, one transcriptional and the other by increase of its stability.
In conclusion we show that DHT decreases ERBB2 expression so we could imagine that androgen deprivation leads to ERBB2 over-expression that could facilitate the growth of prostate cancer. Further experiments will be targeting to determine if androgens influence ERBB2 gene transcription and by what mechanisms.

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Oxygen regulation of tumor perfusion by S-nitrosohemoglobin: implications for radiotherapy.

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Oxygen is needed to fix radiation-induced DNA damages. Several strategies have therefore been developed to transiently increase tumor oxygenation at the time of X-ray delivery. In this context, recent studies in our lab have identified new tumor radiosensitizing approaches. They encompass treatments that allow selective dilation of tumor blood vessels (e.a., by triggering local nitric oxide (NO) production or inhibiting the endothelin-1 vasoconstrictive pathway) and strategies aiming to decrease tumor cell oxygen consumption. Here, we postulated that another target to transiently increase oxygen delivery would be hemoglobin itself, more exactly the S-nitrosylated form of oxygenated hemoglobin (Hb). Indeed, while cell-free Hb has toxic effects (that prevent its use as a blood substitute in humans), S-nitrosohemoglobin (SNO-Hb) has recently been proposed to behave as a nitric oxide (NO) donor at low oxygen tensions. This property, in combination with oxygen transport capacity, suggests that SNO-Hb may have unique potential to reoxygenate hypoxic tumor tissues and, thereby, to potentiate the effects of ionizing radiations.
The present study was therefore designed to test the idea that the allosteric properties of SNO-Hb could be manipulated to enhance oxygen delivery in a hypoxic tumor and circumvent the Hb toxicity. Using Laser Doppler flowmetry, we showed that SNO-Hb infusion to animals breathing air (21% O_2) reduced tumor perfusion without affecting blood pressure and heart rate. Raising the pO_2 (100% O_2 breathing) slowed the release of NO bioactivity from SNO-Hb (ie, prolonged the plasma half-life of the SNO moiety in Hb), preserved tumor perfusion, and raised the blood pressure. In contrast, native Hb reduced both tumor perfusion and heart rate independently of the oxygen concentration of the inhaled gas, and did not elicit hypertensive effects. Dorsal skin window chamber (to image tumor arteriolar reactivity in vivo) and hemodynamic measurements indicated that the preservation of tissue perfusion by micromolar concentrations of SNO-Hb is a composite effect created by reduced peripheral vascular resistance and direct inhibition of the baroreceptor reflex. Overall, these results indicate that the properties of SNO-Hb are attributable to allosteric control of NO release by oxygen in central as well as peripheral issues.
In conclusion, we report here that SNO-Hb can successfully overcome the reduction in tumor perfusion created by Hb. Thus, the O_2-dependent release of NO by SNO-Hb may be exploited to radiosensitize tumors, either directly through a decrease in tumor cell respiration or indirectly through vasodilation-driven reoxygenation.

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Presence of Jamestown Creston Virus in colorectal malignant tumors: A Systematic review of published studies that employed PCR-based methods to detect (JCV) in colorectal carcinomas.

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Introduction : JC virus is a human polyoma virus, which is the etiologic agent of fatal Progressive multifocal leukoencephalopathy (PML). Recently there are reports describing several patients whom PML developed during the treatment with natalizumab. These patients were in clinical trials of natalizumab for multiple sclerosis or Crohn disease and the occurrence of PML was completely unexpected. These reports and subsequent reviews raised the question about presence of JCV in silent stage in human body. Futhermore several studies appeared in medical literature about the presence of JCV in malignant neural and colorectal tumors.
Aim of current study to evaluate the presence of JCV genome in colorectal neoplasms.
I searched the OVID and Pubmed databases for studies that utilized PCR based methods for detecting JCV in colorectal carcinoma samples.
Methods : After excluding the series that lack sufficient data, there remained eight studies that included 445 samples.
Results : Except one study that utilized taqman assay, all studies showed presence of JCV in colorectal carcinomas. Median rate of positivity was %88 (min %26, max %100). Two studies included adenomas (total 36 samples); rates of positivity were %4 and %60. While Presence of JCV in normal colon mucosa was not detected in two studies, another study showed 81 % positivity. One study dealt the presence of JCV in normal mucosa adjacent to tumor and was detected in 43 percent of samples.
Conclusion : Author believes that sufficient evidence exists for concluding that JCV genome presents at least in a considerable portion of colorectal neoplasms. Furthermore it may be concluded that normal gastrointestinal tract might serve as reservoir for JCV, which usually is found in raw urban sewage. We further propose that JCV as an infectious agent might be considered in prevention and treatment in colorectal carcinoma.

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HPV DNA genome presents in the metastases of laryngeal squamous cell carcinomas.

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Introduction : Whereas many studies deal with the HPV and primary lesion, limited number of studies studied the HPV and metastatic disease in head and neck carcinomas. Recently HPV has been considered as causative agent of head and neck carcinomas. The aims of this study were to identify HPV in metastasis from laryngeal carcinoma samples and to describe the physical state of the viral genome to host nuclei.
Methods : Samples from metastatic lesions of primary laryngeal carcinomas were studied. All samples were obtained with cytological means and fixed with alcohol. For assessment of HPV genome presence, chromogenic ISH was performed after cytological review.
Results : Integration of HPV DNA was evidently observed in 22 percent of cases. Of cases, five were from tracheostomy sites and four were from lymph nodes (3 cervical, 1 jugular). No significant correlation was found between tumor differentiation and presence of HPV ($p = 0.8$). HPV genome presence was visualized as dot like signals in the nuclei of tumor cells.
Conclusion : In this study we observed HPV DNA in 22 percent of metastasis of laryngeal carcinomas. ISH study showed a specific punctuate pattern which has been correlated with DNA integration to host nuclei. As our result shown, we think HPV is also involved in progress to metastatic disease in a subset of laryngeal carcinomas.

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Well-differentiated papillary villoglandular adenocarcinoma of the uterine cervix associated with HPV 31-33

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Background: The role of human papillomavirus in the development of squamous cell carcinoma of cervix (SCC) is well known, however pathogenesis of adenocarcinoma of cervix (ADC) is less established. Although HPV DNA is detected in >90 percent of SCC, HPV DNA detection rate is varied between 32 -100 percent in ADC depending on studies. Cervical adenocarcinomas include several different histological subtypes and different histological types are correlated with diverse risk factors. Minimal deviation ADC is associated with Peutz Jeghers Syndrome, clear cell carcinomas is correlated with DES exposure, serous ADC is related with its serous counterpart in endometrium. Of these subtypes, endocervical adenocarcinoma is related specifically with HPV.
Objective: We herein report a case of well-differentiated Papillary Villoglandular Adenocarcinoma which harbors HPV type 31-33. We investigated the HPV presence with chromogenic in situ hybridization and also assessed p53, Ki 67 expression in different component of tumor.
Conclusion: HPV type 33 was detected in the tumor with in situ hybridization, and positive stains for HPV were observed in nuclei of tumor cells. Ki 67 and P53 positivity were observed both invasive and in situ component. We think HPV type 33 infection is likely contributed to the development of this tumor.

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Hepatocyte dysplasia and tumor nodule formation in Cdc42-deficient mouse liver

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The small GTPase Cdc42 regulates many different aspects of cell polarity, such as axon formation in neurons, orientation of the Golgi apparatus in the direction of migration, and directed secretion to the basal side of epithelial cells. To understand the role of Cdc42 in the establishment and maintenance of cell polarity *in vivo*, we generated mice lacking Cdc42 expression in hepatocytes. Mutant mice were born at Mendelian ratios and did not exhibit increased mortality. Histological and ultrastructural analysis, however, revealed severe defects in livers of mutant mice. At age 3 days they showed dramatically enlarged canaliculi between the hepatocytes, although the tight junctions flanking the canaliculi appeared normal, as determined by immuno-fluorescence and electron microscopy. The regular liver plates were completely absent at age 2 months. The normally tight contacts between neighboring hepatocytes were often widened and the E-cadherin expression pattern was changed. Analysis of serum samples indicated a cholestatic phenotype characterized by at least fourfold elevated levels of alkaline phosphatase (AP), tenfold increased concentrations of total and conjugated bilirubin, and increased activities of glutamate pyruvate transaminase and glutamate oxalacetate transaminase (GPT/GOT). After several months we saw signs of liver transformation, such as hepatocyte dysplasia and nodule formation.
We are now trying to histologically and biochemically analyze the molecular mechanisms underlying this phenotype, and to investigate the role of Cdc42 in tumor formation using a liver tumor model and partial hepatectomy.

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Farnesol, an intermediate of the mevalonate pathway, stimulates breast cancer cell growth through the activation of estrogen receptor

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Breast cancer is the most frequent neoplastic disease in the female population of Western countries. It accounts for approximately 30 percent of all diagnosed cancers and approximately 16 percent of all cancer-related deaths. In this clinical context, estrogen receptor (ER) expression is viewed as a reliable prognostic factor, highly predictive of a response to endocrine therapies. Hence, investigations of the mechanism controlling ER levels and activities are of prominent importance. ER acts as an estrogen-modulated transcription factor and can also mediate ligand-independent transcription via crosstalks with growth factors signaling. Moreover, ER may also be a membrane-associated receptor, able of eliciting both genomic and non-genomic responses.
Farnesol (FOH) is a metabolic intermediate of the mevalonate pathway leading to cholesterol synthesis and protein prenylation. It can also bind to farnesoid X receptor (FXR, NR1H4), a member of the superfamily of nuclear receptors / transcription factors that classically heterodimerizes with retinoid X receptors (RXR). FXR is involved in cholesterol metabolism regulation and bile acid transport in liver and gastrointestinal tract, and could be related to cardiovascular diseases. Surprisingly, it is also found in a number of different metastatic cancers.
We assessed the effects of FOH on ER-positive MCF-7 breast cancer cells cultured in steroid-free medium. FOH (50 µM) increased by up to two-fold the growth of MCF-7 cells in a dose-dependent manner, while it had no effects on the ER-negative MDA-MB-231 cells (crystal violet staining assay). In addition, both partial (4-hydroxytamoxifen) and pure antiestrogens (ICI 182,780) completely suppressed the mitogenic effects of FOH in MCF-7 cells, suggesting that they were mediated by activation of ER. Indeed, as documented by Western blot and immunofluorescence, FOH downregulated ER. In these conditions, ER degradation was inhibited by 4-hydroxytamoxifen. FOH also stimulated the transcription of an estrogen-responsive reporter gene, pointing to an estrogenic effect of FOH on MCF-7 cells. In order to explore the mechanism of FOH-induced ER activation, we detected FXR protein in MCF-7 cells, both by Western blot (~60kDa) and immunofluorescence. We are currently looking for the possible occurrence of FXR-ER complexes (ER immunoprecipitation and FXR immunoblot). In addition, other experiments are ongoing to inhibit FXR expression in MCF-7 cells by using FXR siRNA. Use of these FXR-null cells would definitely establish the involvement of this nuclear receptor in the activation of ER by FOH and in FOH-induced mitogenic effects.
In conclusion, our study demonstrates for the first time that FOH stimulates MCF-7 cells proliferation through the activation of ER. We speculate that ER activation could occur through FXR-ER heterodimerization. Thus, FOH, an important intracellular metabolite, could contribute to the basal stimulation of ER in breast cancer cells. Inhibition of FOH production by using inhibitors of the mevalonate pathway (nitrogen-containing bisphosphonates or statins) could reduce FOH-induced ER activation and ER-mediated cancer cell growth.

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Proteomic profiling to search for new biomarkers of tumor-induced osteolysis

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The proteomic analysis, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) coupled with mass spectrometry analysis (MS), allows the assessment of protein profiles by simultaneously examining the expression of hundreds to thousands of proteins. It may determine all coordinated changes at protein levels between clinical groups to generate clusters of markers representative of specific cell functions. This technology should be a useful tool for protein profiling of clinical specimens to search for new biomarkers of tumor-induced osteolysis in serum from patients with bone metastases from breast cancer.
The 2D-PAGE allows the separation of a complex protein mixture from tissues, cells or biological fluids. Serum is a common source of proteins for 2D electrophoresis because of its very high protein and low salt concentrations, simplifying sample preparation (critical part of 2D-PAGE). However, the major drawback of serum samples is the high proportion of albumin (Alb) and immunoglobulin (IgG), which can reach 90% of the total protein content. These major proteins could occult minor proteins, which can be potential candidate biomarkers. In a previous study, we have reported the interest and efficiency of depletion of Alb/IgG from serum samples prior to 2D-PAGE by the comparison of 2D gels of native and depleted serum samples from 20 healthy premenopausal women. The depletion step increases the detection of weakly expressed proteins and therefore provides better conditions for the search of novel biomarkers.
In order to search for new biomarkers of bone resorption due to metastatic cancer cells, we compared serum protein expression profiles from patients with breast carcinomas with or without bone metastases. We analyzed Alb/IgG depleted serum by 2D-PAGE and detected several protein spots in the bone metastasis group. Preliminary analysis suggests that this set of spots includes ceruloplasmin, a protein known to be involved in metastatization process. Other protein changes are under MS analysis.
In addition, in order to study the possible direct resorption of bone by cancer cells, we developed an *in vitro* model of tumor-induced osteolysis by culturing MDA-MB-231 breast cancer cells on bovine dead bone slices. A classical bone resorption marker (the telopeptide CTx) could be detected in the culture medium of MDA-MB-231 cells cultured on bone slices. We are currently searching for potential new biomarkers of bone resorption released into the culture medium.
Moreover, this *in vitro* study permitted to examine the effects of bone matrix components on the MDA-MB-231 cell proteome. In a pilot study, we detected many changes in protein expression profiles between MDA-MB-231 cells cultured on bone slices as compared with incubation on plastic dishes. Significant changes are currently under analysis by MS.
In conclusion, current knowledge emphasizes the necessity to investigate tumor-induced osteolysis through a more global comprehensive approach as provided by proteomic analysis that allows the detection of variations of many factors. Proteomic profiling of serum from breast cancer patients without metastases versus patients with bone metastases could reveal critical biomarkers related to bone degradation directly mediated by breast cancer cells.

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In vitro differentiation of human bone marrow-derived mesenchymal stem cells towards the osteoblast lineage. A model to study the effects of cancer cells on bone formation.

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Breast carcinoma has a high predisposition to metastasize to bone and to induce a marked osteoclast-mediated bone destruction. However, osteoblasts indirectly participate in the process of tumor-induced osteolysis. Tumor cells can activate osteoclasts notably through the secretion of PTHrP that acts on osteoblasts and increase RANKLigand expression that will induce osteoclast differentiation and activity. Osteoblasts are actually pivotal cells for the control of bone turnover. Immature osteoblasts regulate the differentiation and the activity of osteoclasts, while mature osteoblasts produce bone matrix (collagen synthesis and mineralisation).
Human bone marrow-derived mesenchymal stem cells (MSC) are pluripotent progenitor cells that can be differentiated into many mesenchymal lineages, including chondroblasts, adipocytes or osteoblasts, by appropriate stimulations. The effect of cancer cells on osteoblast differentiation is unknown.
MSC were isolated from fresh healthy bone marrow specimens and identified by expression of SH2 and SH3 antigens and the absence of CD45 antigen. Undifferentiated MSC are expanded in αMEM supplemented with 20% FCS without any change in their differentiation potential. MSC were incubated with dexamethasone, ascorbic acid and β-glycerophophate-enriched medium (DAG) to induce MSC differentiation towards the osteoblast lineage.
MSC obtained from 9 donors were incubated in DAG medium during the first four passages. At each passage, MSC were examined after 7, 14 and 21 days of incubation. Alkaline phosphatase (ALP) activity, a classical marker of osteoblastic lineage was reliably increased in MSC incubated with DAG. The increase was already detectable at day 7 (3.3 fold versus untreated MSC), reached a maximum after 14 days of DAG stimulation (10.6 fold) and decreased after 3 weeks of culture (6.5 fold). The increase in ALP activity was low in MSC tested after the first passage, became significant after the second passage and dropped after the fourth passage. In addition, cell-mediated matrix mineralization was determined by von Kossa staining. After the first cell passage, DAG medium stimulated calcium deposition at day 14 and even more at day 21 (15.9 and 24.8 fold versus untreated MSC, respectively) in MSC from two thirds of the donors (6/9). After the second passage, DAG-stimulated matrix mineralization was observed in MSC from nearly all donors (8/9), again at days 14 and 21. However, after the fourth passage, matrix mineralization was stopped in all MSC tested, indicating the loss of differentiation potential of these cells. Furthermore, we are currently testing and comparing several other osteoblastic markers, assessing the expression of genes for early (type I collagen), middle (ALP), and late (osteopontin, osteocalcin, PTH receptor) differenciation markers of the osteogenic lineage by semi-quantitative RT-PCR. We thus observed a 3-fold increase in ALP and osteopontin mRNA in MSC already after 7 days of culture in DAG medium, while PTH receptor was increased for more than 7 fold only after 21 days.
In conclusion, this study describes an *in vitro* model of DAG-induced osteogenic differentiation of bone marrow mesenchymal stem cells, using various osteoblastic markers. Our data indicate the need of use MSC during the second and third cell passages. Human MSC should constitute a useful *in vitro* tool to study the effects of osteotropic cancer cells on osteoblast differentiation and to better understand the reduced bone formation in the process of tumor-induced osteolysis.

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Analysis of mice lacking p120 catenin expression in hepatocytes

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Acquisition of invasive and metastatic capabilities is frequently associated with loss of cell-cell adhesion. One of the major constituents of the cell-adhesion complex in epithelial cells is E-cadherin, a transmembrane protein involved in homotypic cell-cell adhesion. The p120 catenin (p120ctn) protein is a member of the Armadillo-protein family and also a key component of the adherens junctions, where it interacts at the juxtamembrane site with the cytoplasmic tail of the cadherins. New evidence indicates that p120ctn acts as an essential surface retention signal for cadherins and participates directly or indirectly in trafficking decisions that control cadherin turnover at the cell surface. The p120ctn level acts as a sensor of cadherin levels, providing a crucial rheostat or set point mechanism controlling cadherin availability. E-cadherin is generally known as a tumor and/or metastasis suppressor, depending on the mechanism and timing of E-cadherin downregulation.
By crossing mice with a floxed p120ctn allele with albumin-Cre mice, we generated mice lacking p120ctn expression specifically in hepatocytes. Although mice lacking p120ctn in other organs generally display an embryonic or postnatal lethal phenotype, our mutant mice were born at Mendelian ratios and stayed alive for at least five months.
The liver-specific p120ctn KO mice showed an increased liver weight. In those livers a ductular reaction was observed on H/E stainings. Immunostaining showed a changed E-cadherin expression profile and reduced N-cadherin levels. Analysis of serum samples indicated elevated levels of alkaline phosphatase (AP) and tenfold increased concentrations of total and conjugated bilirubin, whereas glutamyl pyruvic transaminase (GPT) and glutamyl oxaloacetic transaminase (GOT) levels remained unchanged.
Further analysis of the in vivo roles of p120ctn in the liver and of the possible relevance of its loss for the causation of hepatocellular carcinoma (HCC), one of the most common solid tumors worldwide, is in progress.

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Thyrotropin-stimulated primary thyroid cell cultures as a model of benign thyroid tumors: a microarray analysis

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In a large number of tumors from various origin, mutations have been detected in genes that are involved in the normal regulation of cell division. Since these genes play a critical role in the decision of the cell to divide, they are putative oncogenes and anti-oncogenes. In the thyroid, cell growth and proliferation are induced by the binding of thyrotropin (TSH) to the TSH receptor, which leads to the activation of the cyclic AMP (cAMP) signaling cascade. Previous research has shown that mutations in components of the cAMP-cascade promote unrestrained growth in thyroid cells, resulting in the formation of benign thyroid tumors. In this study, primary culture of human thyrocytes were used as a model to search for genes that might be involved in the induction and promotion of a thyroid tumor. Cultures were treated with TSH to investigate the proliferative intracellular signaling pathways that are triggered by this hormone. Gene expression profiling was performed by cDNA microarray analysis (23,232 spots) after stimulation of the cultures for different times (1.5 hours, 3 hours, 16 hours, 24 hours and 48 hours). Comparison of gene expression in primary cultures in the presence or absence of TSH demonstrated an upregulation and downregulation of a large number of spots at all the time points investigated. The reproducibility of the experiments was high: duplicate microarrays, intensity ratios of different spots corresponding to the same genes on the same microarray, as well as the kinetic evolution, was consistent throughout different experiments. The pattern of expression shows an early shift in the transcription factors and signal transduction proteins expressed, i.e. a change of cell program. Later changes reflect a stimulation of specific cell functions and a trophic effect. This pattern of late changes reflects in part the pattern of autonomous adenomas. A number of genes were selected to confirm modulation by using real time PCR. To conclude, primary culture of human thyrocytes stimulated with TSH gives insight in the signaling pathways that are involved in mitogenic cell signaling and provides a model for autonomous adenomas.

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Gene Expression in Thyroid Papillary Carcinomas.

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Thyroid tumors are the most frequent endocrine tumors. The major phenotypes are the benign adenomas, autonomously hyperfunctioning or not, and the malignant carcinomas. The main malignant thyroid tumors are subdivided in follicular or papillary (PTCs) carcinomas, still partly differentiated, and both of which may evolve in anaplastic carcinoma, totally dedifferentiated. There has been a considerable increase in thyroid cancer after the Chernobyl nuclear power plant accident. These thyroid cancers have been identified as PTCs on the basis of their pathology. The Chernobyl accident therefore provides a unique opportunity to characterize radiation-induced thyroid cancer.
The aim of this work was to characterize the molecular profile of PTCs, using the cDNA microarray technology, and to ask whether the post-Chernobyl PTCs could be distinguished from sporadic PTCs, ie. if both have the same molecular phenotype. A molecular signature allowing to separate both types of PTCs is presented and discussed.