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Activation of the endothelin axis in osteoblasts: genomic analysis and identification of biomarkers of osteoblastic metastasis

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Osteoblastic metastases are common in advanced prostate cancer and some cases of breast cancer. It has been shown that uncontrolled bone formation at the site of metastasis is caused by a number of growth factors, in particular endothelin-1 (ET-1). It has been proposed that secretion of ET-1 by prostate cancer cells is a major factor in formation of osteoblastic metastases. A highly specific antagonist of the ETa receptor, ABT-627, has been developed by Abbott for treatment of metastatic prostate cancer.
To gain better understanding of the mechanism of ET-1-induced osteoblast proliferation and to identify biomarkers of osteoblastic metastasis, we studied the effects of ET-1 (±ABT-627) on primary human osteoblasts and mouse osteoblastic cells by measuring the phosphorylation of several pro-growth kinases and by determining transcriptional activation on a genome-wide scale.
We observed rapid phosphorylation of p38, ERK1/2, and JUNK kinases after the ET-1 treatment. These effects were completely blocked by ABT-627. To determine the pathways involved in the ET-1-induced osteoblast proliferation, we used microarray profiling combined with systematic pathway analysis. We observed coordinated induction of genes associated with invasion/metastasis, survival, and osteoblast and osteoclast maturation. Nearly all the genes induced by ET-1 were blocked by ABT-627, implying that all the effects of ET-1 are mediated by the ETa receptor.
The ET-1 signature in osteoblasts contained several genes coding for secreted proteins previously implicated in invasion and metastasis. Their secretion was confirmed by ELISA. These proteins are currently being explored as biomarkers for osteoblastic metastasis and efficacy biomarkers for ABT-627.
Our studies support and refine the existing model of cancer cell/osteoblast interactions in osteoblastic metastases. They also yielded several candidate biomarkers that can potentially be used to monitor the progression of osteoblastic metastases and the efficacy of antimetastatic therapies.

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The Receptor Activator of Nuclear Factor–kB Ligand (RANKL)/Osteoprotegerin (OPG) axis is severely disrupted in patients with solid tumours and osseous metastasis

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Background: The pathophysiology of bone metastasis has not been fully clarified in several solid tumors. The receptor activator of nuclear factor-kB (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) system has a major role in osteoclastogenesis. Recently, special emphasis has been given in determining the possible mechanisms by which this system affects the process of bone remodeling in cancer. We investigated the role of these molecules along with novel markers of bone turnover in patients with solid tumors metastatic to bone in relation to the type of malignancy and the neoplastic burden to the skeleton.
Material and methods: Blood specimens from 40 patients with breast, lung and prostate cancer metastatic to the skeleton were collected at the time of diagnosis. Levels of soluble RANKL (sRANKL), OPG, sRANKL/OPG ratio, 5b isoenzyme of tartrate resistant acid phosphatase (TRACP-5b), C-terminal cross-linked telopeptide of type I collagen (CTX), C-terminal propeptide of procollagen type I (PICP), osteopontin (OPN), bone alkaline phosphatase (bALP) and osteocalcin (OC) were assessed using enzyme-linked immunosorbent assay (ELISA) and were compared to those of an age and sex-matched healthy control group.
Results: Patients with breast or lung cancer and bone metastasis had elevated serum levels of osteoclastic activity markers, such as sRANKL ($p = 0.004$), TRACP-5b ($p = 0.022$) and OPN ($p < 0.0001$), compared to controls. Furthermore, sRANKL correlated with the extend of metastatic bone burden ($p = 0.035$ in patients with more than ten sites of osseous metastasis). Levels of osteoblastic activity markers were also significantly elevated compared to controls, including OPG ($p < 0.0001$), bALP($p \leqslant 0.0001$) and OC ($p \leqslant 0.0001$) but not PICP ($p = 0.26$). In tumor specific analysis, prostate cancer follows a different pattern of bone remodeling with excessive increase of osteoblast function, as reflected by elevated values of OPG ($p = 0.0034$) and bALP ($p = 0.002$) but without any significant change of bone resorption markers such as sRANKL, TRACP-5b, CTX and OPN.
Conclusions: These results suggest that enhanced osteoclastic activity in patients with solid tumors metastatic to bone is mediated through severe disruption of the sRANKL/OPG axis in bone marrow microenvironment. Breast and lung cancer seem to exert their osteolytic action through upregulation of the sRANKL/OPG system, whereas prostate cancer seems to provoke profound elevation of OPG levels only, resulting in increased osteoblastic activity.

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Upregulated claudin-1 and downregulated claudin-3 and claudin-4 expression differentiates human hepatocellular carcinoma from colorectal liver metastasis

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Background: Tight junction proteins as claudins, occludin and JAM or peripheral zonula occludens (ZO) proteins are widely implicated in carcinogenesis. Claudins (1–24) have been recently identified as integral proteins of tight junction strands. Claudin-4 has not been found in normal hepatocytes or bile duct epithelium. Further, downregulated claudin-1 is associated with poor prognosis of colon carcinoma. Claudin-3 and -4 can function as the receptor of the Clostridium perfringens enterotoxin. Aim: The objective was to characterize the expression of claudins 1–4 and -7 in human HCCs and colorectal liver metastases compared with corresponding nontumorous and normal liver samples.
Material and methods: 19 human hepatocellular carcinoma (HCC) and 12 colon metastasis samples were examined by real-time RT-PCR and immunohistochemistry for expression of claudins-1–4 and -7. Relative quantification utilized GAPDH and beta-actin as internal control genes. The HCC samples did not show nuclear staining for beta-catenin indicating activation of the wnt pathway.
Results: Claudin-4 in HCCs was found downregulated 9.7 folds and 43 folds compared with normal livers and metastases, respectively. Claudin-3 in HCCs showed 5.5 fold downregulation in comparison to normal liver, however, there was 11 fold expressional downregulation compared with the metastases. Claudin-1 on the other hand showed 3.3 fold mRNA upregulation in HCCs compared with metastases. Immunohistochemistry predominantly detected membranous staining pattern and confirmed RNA expression data. Western blot analysis showed no expression of claudin-4 in HCC, however, immnohistochemistry detected the presence of claudin-4 on the bile ducts in nontumorous tissue.
Conclusion: Taken together, claudin-3 and -4 expressions were markedly downregulated in HCCs contrary to metastases, while claudin-1 showed higher expression in primary HCCs. Claudin-4 expression was localized to the bile ducts. These findings might help to differenciate hepatocellular carcinoma from colorectal metastasis. Biological role and significance of upregulated claudin-1 and downregulated claudin-4 in the progression of HCC should be elucidated.
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Polymorphisms as predictive markers

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A single nucleotide polymorphism in the CCND1 gene is associated with decreased risk of developing metastases in female breast cancer patients

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Background: One of the key regulators of the G1 phase in the cell cycle is Cyclin D1 (CCND1). In addition, CCND1 is an important factor in tumor development and progression. A common 870 G > A polymorphism in the gene for CCND1 has been linked to alternative splicing and cancer susceptibility. The objective of this study was to evaluate the role of this polymorphism for breast cancer metastazing.
Material and methods: A retrospective analysis including 500 female breast cancer patients was performed. In a Cox regression model including CCND1 870 A carriage, age at diagnosis, primary presence of regional