patients with epithelial circulating cells, which might indicate a capacity of these malignant cells to invade blood vessels.
Supported by Fapesp.

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Development of a measles virus vector targeting breast cancer cells by expression of single chain antibody against HER-2/neu

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Background: HER-2/neu is overexpressed in 25% of breast cancers, and is associated with poor prognosis. In order to develop effective therapies for breast cancer, we used a novel virus vector targeting the neu protein. We previously established a reverse genetics system for measles virus (MV) using the MV-HL strain. By using this system, we have previously constructed a recombinant MV (rMV) that expresses a single chain antibody (ScFv) against human alpha-fetoprotein (AFP). This recombinant virus inhibited colony formation of AFP-positive human hepatoma cells. In the present study, we constructed rMV expressing ScFv against activated rat HER-2/neu protein to be tested *in vivo* in a transgenic mouse model of spontaneous breast cancer.
Materials and Methods: We constructed a cDNA in which the ScFv against rat HER-2/neu is fused with the transmembrane domain (TMD) of vesicular stomatitis virus (VSV)-glyco (G) protein, and inserted it as an additional transcription unit between the N and P genes of the MV genome. We then rescued the virus (rMV-aneu), and investigated the ability to replicate in breast cancer cells and the potential as an antitumor agent.
Results: We succeeded in rescuing the rMV-aneu from the construct using the reverse genetics system. The rMV-aneu replicated as well as the parent MV in B95a cells, derived from marmoset B lymphoma. The rMV-aneu grew in N2C cells, a mammary carcinoma cell line established from a spontaneous BALBneuT tumor expressing rat HER-2/neu on their surface, whereas the parental MV did not show any infectivity. The protein produced by the inserted gene within the recombinant MV was properly expressed in the infected N2C cells. The rMV-aneu significantly reduced cell viability as measured by the metabolic activity of the infected cells. In contrast, the parental MV and mock infection did not cause any change in the activity. The effects of the rMV-aneu on the rat neu transfected human cells and on the transformed cells *in vivo* are currently under investigation.
Conclusions: As an approach to develop tumor-targeted, replication-competent viruses useful in breast cancer treatment, we constructed an rMV that express ScFv recognizing activated HER-2/neu. The insertion of the ScFv gene fused with G-TMD into the rMV genome induced its infectivity. The rMV-aneu significantly inhibited the HER-2/neu+ cell activity *in vitro*. These results suggest the possibility that the rMV-aneu may be useful in breast cancer therapy.

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Association between serum estrogen and androgen concentrations and tumour receptor status in postmenopausal breast cancer

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Elevated levels of endogenous sexual hormones; estrogen (E2), estrone (E1), testosterone (TE) and their precursors; androstenedione (AD), dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEA-S), E1 sulphate (E1-S), have been associated with the risk of breast cancer in postmenopausal women. In this study we investigate the correlation between serum hormone concentrations and tumour receptor status. Besides the levels of sexual hormones and precursors, sex hormone-binding globulin (SHBG), insulin-like growth factor-1 (IGF-1) and IGF binding protein-3 (IGFBP-3) were also measured by fully automatized equipment using RIA and IRMA methods. The estrogen (ER) and progesterone receptors (PR) expression in tumour tissues were determined by ICH, and MedCalc Software was used for statistical analysis.
Our study involved 444 postmenopausal patients with primary breast cancer of Stage I, II prior to surgical intervention and 250 healthy controls [average age in both groups was 64 years]. 358 of cancer patients were diagnosed for invasive ductal carcinoma (DC), 55 for invasive lobular carcinoma (LC), 29 for DC in situ (DCIS), and 2 for LCIS. 297 were ER and PR-positive [ER+/PR+], while 78 were ER and PR-negative [ER−/PR−]. Significant increase of E1, E1-S, AD, TE, DHEA, DHEA-S levels and significant decreases of SHBG level in cancerous cases were found by Mann-Whitney statistical analysis.
The median value of serum E1, AD, E1-S, IGF-1, E2 and TE were higher in patients with [ER+/PR+] receptor status than in patients with [ER−/PR−] receptor status. In E1 ($p < 0.0003$), AD ($p < 0.0009$) and E1-S ($p < 0.0096$) levels the difference was highly significant. Patients were rank ordered according to the increasing serum E1, E1-S and AD concentrations. In the highest quintile of each series 74–80% of patients were [ER+/PR+], while 9–11% of them were [ER−/PR−]. Using logistic regression analysis, the probability of tumour receptor positivity can be predicted based on the knowledge of serum hormone (E1, E1-S, AD) levels. In addition, E1-S and TE levels tend to be markedly associated with invasive DC (DC vs. LC, $p < 0.0109$ for E1-S; $p < 0.019$ for TE).
Our study supports the hypothesis that the circulating sex steroid hormone levels are strongly correlated with risk of [ER+/PR+] breast tumours.
Some kits for IGFBP-3, E1-S, AD and MedCalc software were gratefully donated by Laborexpert Ltd., Budapest, Hungary.

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The prognostic influence of Plasminogen Activator Inhibitor-1 in early breast cancer is not related to estimates of angiogenesis

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Introduction: Plasminogen Activator Inhibitor type-1 (PAI-1) is one of the key proteases involved in tumour invasion and microenvironment remodelling. Indeed, high levels of PAI-1 have been associated with poor prognosis in several tumour types. In several experimental studies PAI-1 has been shown to play a role in angiogenic processes, and since estimates of tumour angiogenesis have been demonstrated as predictors of poor prognosis this study investigates the relationship between estimates of tumour angiogenesis and protein levels of PAI-1 in breast cancer.
Materials and methods: Tumour specimens from 438 patients diagnosed with primary unilateral non-metastatic breast cancer were used. Median follow-up was 9.5 years, and 168 patients (38%) had died from cancer. Angiogenesis scores were performed on paraffin-embedded tissue slides stained with anti-CD34, and vessels were counted using a Chalkley grid in hot spots. Protein levels of PAI-1 were measured in supernatants from frozen tumour tissue using a sandwich ELISA kit with monoclonal catching and detecting antibodies.
Results: Median Chalkley count was 5.00 (range, 2.67–12.00), and median PAI-1 level was 0.70 ng/mg protein (range, 0–90 ng/mg protein). Chalkley counts were not correlated with PAI-1. Both high Chalkley counts and high PAI-1 were significantly correlated with high malignancy grade and lack of estrogen receptor, and high Chalkley counts furthermore correlated with large T size. High Chalkley counts and PAI-1 in tertiles were both correlated with poor disease specific survival (DSS) ($P = 0.002$ and $P = 0.05$, respectively). Combining low/low versus high/high tertiles of Chalkley counts and PAI-1 showed actuarial survival rates of 75% versus 52%, respectively ($P = 0.0008$). In multivariate analysis high N-stage ($P' < 0.0001$), grade ($P < 0.0001$) and increasing levels of PAI-1 ($P = 0.004$) were identified as independent markers of cancer-death.
Conclusions: In univariate analysis both Chalkley counts and PAI-1 levels were associated with poor DSS. Combining lowest versus highest tertiles of both factors separated the patients into groups with significantly different survival. This study suggests that the prognostic impact of PAI-1 is independent of its supposed involvement in angiogenic processes.

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Gene expression associated to response to doxorubicin based primary chemotherapy in breast cancer

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Background: This study was undertaken to identify genes that could predict response to doxorubicin based primary chemotherapy in breast cancer patients.
Patients and methods: Patients (pt) with confirmed invasive breast cancer on samples obtained by core or incisional biopsy, clinical stages (CS) II or