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NOEY2 (ARHI) mRNA expression in human breast cancer tissue

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Introduction: NOEY2 (ARHI) is a putative maternally imprinted tumor suppressor gene, which encodes a new member of the Ras superfamily of small G protein. NOEY2 is expressed consistently in normal ovarian and breast epitherial cells but is down-regulated frequently in ovarian and breast cancers. In this study, we have examined NOEY2 mRNA expression in human breast cancer tissue and compared with clinicopathological facters.

Material and Method: Breast cancer tissue and non cancerous tissue in surgical specimens were evaluated from 110 patients with breast cancer. Relative mRNA expression of NOEY2 in each samples was assessed by real-time quantitive RT-PCR analysis.

Result: Reduction of copy number of NOEY2 mRNA in cancer tissue was found in 61/ 110 (55%) patients. NOEY2 expression was more reduced in high nucleal grade tumors and lymph node positive patients, but this difference was not significant. There was no correlation between NOEY2 mRNA expression and other clinicopathological factors (tumor size, age, hormon receptor status). Conclusion: NOEY2 is down-regulated frequently in human breast cancer tissue and independent of standard clinic

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Adoptive immunotherapy of malignant effusions

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The data obtained from 21 breast cancer patiens with chemoresistant malignant pleural effusions were submitted to a pilot study with intrapleural (ip) recombinant interleukin-2 (IL-2) and lymphokine activated killer (LAK) cells administration. All patients were subjected to two or four courses of IL-2 and LAK. The patients were given 0.5–1.0 mln IL-2 (Proleukin) per day ip for 5 days course and twice 100–300 mln LAK ip every week. The LAK generation by IL-2 have been obtained from malignant effusion mononuclear cells with presence of IL-2. In this study, patients response (CR+PR) was 91%. IL-2/LAK-therapy were well tolerated and no interruption occurred. After ip immunotherapy main supopulations of blood lymphocytes was increased< aspeciolly actyvated T-cells (CD3+, CD25+) and NK-cells (CD16+). The tumor cells were disappeard from malignant effusions usually after 1–2 courses of IL-2/LAK-therapy. These data suggested the opportunity to initiated large prospective randomized trail using IL-2/LAK-therapy in patients with malignant effusion.

73 POSTER

Immune disorders induced by anaesthesia and surgery in ovarian hormone suppression for breast cancer. Comparison between laparatomy and laparoscopy

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Surgery and general anesthesia determine modifications of the immune system. As laparoscopy is still considered an issue for evaluation in the case of patients with cancer, the purpose of this research was to compare post-surgical immunological alterations determined by anesthesia and surgery for ooforectomy in open vs. laparoscopic surgery. It is a prospective, randomized study performed on patients with breast cancer and indication for surgical hormone suppression: 60 patients have been submitted to open (classical) surgery; 58 patients have had laparoscopic surgery. The two groups were similar from the point of view of: -age, -stage of the disease, -associated morbidity, -breast cancer treatment, -type of general anesthesia and surgical time length. Evaluation was performed before and 24 hrs, respectively 72 hrs after surgery. The immunological parameters studied were: -Lymphocyte blastic transformation test (LBTT); -Determination of immunoglobulins, Interleukin-6 and Interleukin-2, and C reactive protein; absolute number of lymphocytes; serum protein electrophoresis has been performed

Results: Before surgery, it was found in both groups a decrease in the number of lymphocytes, decrease of the LBT index to Phytohemaglutinin and increase of that index to Concanavalin A; IL-2 production in lymphocyte cultures of 32pg/0,1ml, and a serum level of 4.1 pg/ml IL6. In the group submitted to classical surgery, an increase in the leukocyte and a decrease in the lymphocyte count were found after surgery. In the group with laparoscopic surgery, there was an increase in the number of leukocytes, while the lymphocyte proportion remained constant, i.e. at the pre-surgery level; LBT index decreased from 7.3 to 6.1 in the classical interventions and from 7.3 to 6.9 in the laparoscopic ones. In the patients with classical surgery, IL-2 concentration in lymphocyte cultures was undetectable, while in the group with laparoscopic surgery IL-2 levels were 30pg/0,1ml. IL-6 in the classical group rose to 7.1 pg/ml, while in the laparoscopic group it rose to 5.9 pg/ml. This is correlated with the increase of the C reactive protein from 6 mg/l to 18 mg/l - in the classical group, and to 10 mg/l - in the laparoscopic one.

The analysis of the results leads to the conclusion that laparoscopic surgery induces fewer immunological disorders than open surgery, an element of importance in the case of a disease with immunological implications in its pathogenesis and evolution.

74 POSTER

Crude catechin regulated the cdc2 phospholylation in breast cancer cells MDA-MB-231

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Crude catechin (catechin) included 60% of epigallocathechin-3-gallate (EGCG). It was reported that EGCG inhibited the breast cancer cell growth and prevented the breast cancer. However, the mechanisms of the cell growth inhibition or prevention of the cancer by catechin is still unclear. In this study we analysed the growth suppressive effect of catechin on human breast cancer cell line MDA-MB-231. Compared to untreated cells, treated with catechin showed significantly dose-dependent growth inhibition. The effect of catechin on cell cycle progression, catechin treated cells showed a 8.6% increased of G2/M phase. Treatment of MDA-MB-231 for 48h with 100mocroM catechin, expression of phospholylated cdc2 protein decreased significantly. Our data suggested that the antiproliferative activity of cathechin growth-suppressive status in G2/M phase, possibly through regulation of cdc2 phospholylation. The presently demonstrated specific mechanisms of cell cycle is potential epigenetic molecular targets for breast cancer treatment or prevention by catechin.

Wednesday, 20 March 2002 16:30–18:00

PROFFERED PAPERS

Imaging the sentinel node

75 ORAL

Lymph node metastases detection by FDG-PET and sentinel node biopsy in breast cancer patients: comparison of these different approaches

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Background: Axillary dissection (ALND) for detection of metastatic involvement is used to plan adjuvant treatments for breast cancer (BC) patients. ALND is a costly procedure with various side effects.80% or more of T1 patients are node negative and ALND is useless. Recently, sentinel node (SN) biopsy has been suggested as method of reference for the evaluation of regional nodal metastases and for the decision on the need of a ALND. SN biopsy is an invasive approach, with a not negligible risk of false negative results. Conversely, Positron Emission Tomography (FDG-PET) is a non-invasive repeatable method able to evaluate all the regional nodes in BC: our PET experience on nodal involvement in BC has given interesting data of sensitivity and negative predictive value, comparable with SN

biopsy. The aim in this work is a comparison between the two methods in the same series.

Methods: T1N0 BC patients were studied. FDG-PET has been performed no later than 48 hours before surgery. Lymphoscintigraphy (LS) has been performed within 6 hours before surgery. After breast surgery, radioguided biopsy of the SN has been performed followed in all cases by ALND. Metastatic involvement of the SN and the other non-SN has been evaluated on definitive sections and was the basis of the comparison between the two methods.

Results: 35 patients have been studied,median age 56 years(range=39-70). All patients had pT1 BC except 3 pT2 (size less than 2.5 cm). The median histological tumor size was 13 mm(range=2-23 mm). All lymph nodes detected by LS were in axillary region, and detection rate was 100%. All SN were identified with intra-operative gamma probe, and then biopsied. All patients underwent ALND (on the average,17 lymph nodes surgically removed).15 patients showed nodal metastases. The SN biopsy results showed 3 false-negative (2 partial and 1 embolic involvement detected in non-SN), whereas FDG-PET failed to detect 4 axillary involvement (2 microembolic,1 partial and 1 pluriembolic); one patient with partial nodal involvement was undetected both the methods. No false positive FDG-PET scan was seen.

Conclusions: This is the first study comparing these two different methods on the same series. The preliminary results suggest a similar sensitivity, thus giving a contribution to a further statement on validity of FDG-PET for evaluation of BC regional node involvement.

76 ORAL

Does ultrasonographic examination of the axilla in the preoperative screening of breast cancer patients reduce the number of sentinel lymph node procedures?

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Purpose: Currently most breast cancer patients with a clinically negative axilla will undergo a sentinel lymph node (SN) procedure. The aim of this study was: 1. To investigate if the combined use of preoperative axillary ultrasound and Fine Needle Aspiration (FNA) reduces the number of SN procedures. 2. To identify ultrasonographic characteristics that predict metastatic invasion of a lymph node.

Patients and Methods: All breast cancer patients with a clinically negative axilla eligible for a SN procedure (N=268) were included. FNA was performed on lymph nodes with a smallest diameter larger of equal to 5 mm. The number of patients with visible nodes, the number of aspirated nodes and their diagnosis were noted. The SN procedure was bypassed if a tumor-positive node was found by cytological examination. The reduction in sentinel node procedures was calculated by dividing the number of tumor-positive FNAs by the total number of axillas.

Appearance of cortex and hilus were noted; length, width, and cortex thickness of all aspirated nodes were measured and the shape of these nodes (i.e., length/width ratio) was calculated.

Results: In 93 patients (35%) at least one lymph node was detected in the axilla by ultrasound. FNA was performed on 66 nodes; 37 (56%) showed tumor cells. For the whole group a reduction in sentinel node procedures of 14% was obtained, at the expense of FNA in 25% of the patients. Thirty-one percent of all patients with metastases in the axilla were detected with ultrasound and FNA.

Linear discriminant analysis and step-wise selection of nodal features showed that cortex thickness is the main feature to predict the presence of metastasis (AZ=0.89). Prospective estimates indicate that performing FNA on all lymph nodes with a cortex thickness of at least 2.7 mm will reduce the number of SN procedures with 14%, thereby performing FNA in only 18% of the patients.

Conclusion: Preoperative ultrasonography of the axilla reduces the number of SN procedures. Cortex thickness is the main feature to predict metastasis.

77 ORAL

Selective axillary surgery in breast cancer patients based on positron emission tomography with 18f-fluoro-2-deoxy-d-glucose: not yet!

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Introduction: Positron emission tomography (PET) with 18F-fluoro-2-deoxy-D-glucose (18F-FDG) has been shown to be a sensitive and specific diagnostic tool for detection of axillary lymph node macro-metastases in women with breast cancer [1]. The aim of this study was to evaluate the capability of PET to detect micro-metastases (<2mm).

Methods: In a prospective study we evaluated 31 patients with invasive pT1 and pT2 breast cancer. Preoperative 18F-FDG-PET for detection of axillary lymph node metastases was performed. After PET imaging, all patients underwent tumorectomy or mastectomy, sentinel lymph node [SLN] procedure, and axillary lymph node dissection, if metastases in the SLN were found. Results of PET imaging were compared to histopathologic analysis (hematoxylin-eosin-staining [H&E], immunohistochemistry [CK22, Lu-5], step sections of lymph nodes).

Results: Seventeen patients (55%) had no metastases in the SLN. Examination of the SLNs of the remaining 14 patients showed 9 macrometastases, 4 micrometastases and once disseminated single carcinoma cells. The overall sensitivity, specificity and negative predictive value of PET in the detection of axillary lymph node metastases were 43%, 94% and 67%, respectively.

Results of PET imaging (n=31)

	No. of pts.	Description
True +	6	6 macrometastases (3mm*, 7mm, 8mm, 11mm, 22mm, 30mm)
True –	16	Negative by H&E and immunohistochemistry
False +	1	Non-Hodgkin-lymphoma (14mm)
False –	8	3 macrometastases (4mm, 4mm, 13mm)
		4 micrometastases (0.1mm, 0.12mm, 0.8mm, 1mm)
		1 disseminated single carcinoma cells

*Smallest detected metastasis by PET

Conclusions: Our data indicate that PET imaging with 18F-FDG does actually not provide the spatial resolution necessary to accurately assess the axillary lymph node status in patients with small metastases. All micrometastases and several macrometastases up to 13mm of diameter have not been identified. Therefore, the time has not yet come for selective axillary surgery in breast cancer patients based on 18F-FDG-PET.

References

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78 ORAL

Lymphoscintigraphic detection and selective biopsy results of internal mammary sentinel nodes in breast cancer patients

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Sentinel Node (SN) biopsy has become an alternative to axillary dissection. Preoperative lymphoscintigraphy sometimes reveals SN at the internal mammary (IM) basin. We report on a single institution prevalence and factors associated with scintigraphic appearance of IM SN, as well as on the technique and the results of selective IM SN biopsy.

Patients and Methods: From October 1997 through September 2001, 261 consecutive T1-T2, N0 patients underwent succesful SNB. Peritumoural injections of Tc-99m albumin colloid were used. Multiple view lymphosintigraphy was done at least 2 hrs p.i. Both axillary and IM SNs were selectively biopsied with a gamma probe. Histopathologic study of SNs included serial sectioning and stainig with H&E and cytokeratin.

Results: Lymphoscintigraphic drainage to IM SNs was seen in 58 of 261 pts (22%). As oposed to the 203 pts without IM SN, pts with IM drainage tended to be younger (52 vs 59 y, $p<0.001$), to have more SNs (2.8 vs 1.5,

p<0.001), and to be seated in central quadrants (53% vs 32%, p<0.001). Other variables were not significantly different. When attempted, IM SN biopsy was successful in 98% of the pts (48/49). There were no major complications, and only two minor bleeding episodes were seen. 24 pts presented with negative SNs both at the IM and axillary basins. 10 pts had a positive axillary SN and a negative IM SN. 10 pts had both positive axillary and IM SNs. 4 pts had a positive IM SN and a negative axillary SN. Altogether, IM SNs were positive in 14 pts (29%).

Comment: Using periturnoural injection and preoperative lymphoscintigraphy, IM SNs can be identified in 22% of breast cancer pts. Such pts tend to be relatively young, have central quadrant lesions and display a complex pattern of drainage to SNs (axilla and IM). IM SN biosy is feasable and has low morbidity. Important prognostic information can be derived from such procedure. Also, IM radiotherapy can be offered on a selective basis.

79 ORAL

Elastic scattering spectroscopy for intraoperative detection of sentinel lymph node metastasis

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Background: Intra-operative assessment of sentinel lymph node (SLN) enables the surgeon to decide on immediate axillary lymph node dissection at the time of SLN biopsy. The aim is to develop a device using the principles of Elastic Scattering Spectroscopy (ESS) capable of intraoperative detection of SLN metastasis in patients with invasive breast cancer.

Methods: ESS involves the spectral analysis of light scattering by intra- and extra-cellular components, and has previously been demonstrated to be sensitive to morphological changes of tissue.

The ESS system consists of a white light source, a parallel pair of optical fibres, a spectrometer and a computer. The process involves the delivery of light from the light source directly onto the sectioned surface of LN via one of the optical fibres. After having been scattered by the tissue, the light is collected by the second optical fibre, and is analysed by the spectrometer and the computer, generating an optical spectrum. The entire process takes less than one second.

The optical spectrum was further analysed against a larger and independent training set of optical spectra from LN using model based analysis to determine the status of LN. The result was compared the histological findings.

Results: In total, 75 LN were tested. ESS provided diagnosis in 72 LN (3 LN were deemed indeterminate), and was correct in 62 LN. ESS was able to correctly identify metastasis in 16 out of 19 LN compared with histology. There were 7 false positives and 3 false negatives, thus giving a sensitivity of 84.2% and specificity of 86.8%.

Conclusion: Current intra-operative techniques to assess SLN are imprint cytology and fresh frozen section, which are time consuming and operator dependent. ESS has the potential to provide instant and non-operator dependent intra-operative analysis of SLN in patients with breast cancer; sensitivity and specificity should increase as the database of correlated biopsies increase in size.

80 ORAL

Lymphoscintigraphy in breast cancer- which radiopharmaceutical to use- comparison of two particle sizes (ALBU-RES and Nanocoll)

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Aim: The aim of the study was to evaluate and compare the visualisation of sentinel nodes in lymphoscintigraphy in breast cancer using two radionuclides.

Patients and Methods: The prospective observational study included 269 consecutive patients with clinical stage T1- T2, N0 breast cancer, who underwent lymphatic mapping and sentinel node biopsy using a 2-day protocol. Lymphoscintigraphy was performed four hours after an intratumoral injection of 80-100 MBq 99mTc labelled human albumin colloid with particle size of 0.2-3μm, (ALBU-RES) in 136 patients (group A) or 99mTc albumin microcolloid with particle size of less than 80nm (Nanocoll) in 133 patients (group N) in a volume of 0,2ml. Anterior and lateral views were obtained using a gamma camera. The intensity of uptake in the nodes was classified as weak, clear, strong or absent.

Results: The lymphoscintigraphy showed sentinel nodes in the axilla in 114 of 136 patients (84%) in group A. The median number of the visulised nodes in the axilla was 1 (range 1- 4) in these 114 patients. In group N, 118 of 113 patients (89%) had a median of 2 (range 1-5) hot spots in the axilla (p<0.0005 for the median number of visualised nodes between the patient groups. The proportion with clear or strong uptake of the tracer in the axilla was 66% in group A and 79% in group N (p<0.05). Sentinel lymph nodes in the internal mammary basin were visualised in 18 (13%) patients in group A and in 27 (20%) in group N (p=ns).

Conclusion: More numerous and intense hot spots were visualised in the axilla when using smaller particle size (Nanocoll) pharmaceutical.

Wednesday, 20 March 2002

POSTERS

Sentinel node – technique, diagnosis and management

81 POSTER

Sentinel node biopsy using small-sized Tc-99m Tin colloid

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Objective: The optimum particle size of pharmaceuticals for sentinel lymph node biopsy (SLNB) is still one of the major problems in SLNB. Small-sized particles move easily, but also pass through the SLNs rapidly. On the contrary, large particles move slowly to the SLNs, but stay there for longer. We produced small-sized tin colloid particles and evaluated the clinical usefulness of these particles.

Patients and Method: Patients with no axillary lymph node swelling were recruited to this trial. We produced small-sized tin colloid particles (200-400 nm) by mixing sodium pertechnetium-99m and SnCl2 at the ratio of 1:4, instead of the usual 1:1 ratio. SLNB was performed by injecting 0.5 ml of 74 MBq/ml of this solution at three points peritumorally and 0.2 ml subcutaneouly just over the tumor the day before the operation. Isosulfan blue was also injected peritumorally just before the operation. SLNB was performed using a gamma probe, and a back-up dissection was also performed.

Results: SLNB using the small-sized Tc-Tin colloid particles was performed in 50 patients (SC group). Seventy-four patients in whom the biopsy was performed using large-sized tin colloid particles were examined as controls (LC group). The number of SLN identified and the identification rate were 1.9 and 79% in the LC group, and 3.3 and 100% in the SC group, respectively. The false-negativity rate was 9.6% and 5%, respectively, in the two groups.

Conclusion: Small-sized Tc-99m tin colloid particles are more useful for SLNB as compared to large- sized tin colloid particles.

82 POSTER

Ultrasound guided fna of the axillary lymphnode: a preoperative staging procedure in primary breast cancer

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Currently, the axillary lymphnode dissection is being replaced by the sentinel node procedure. This method is time-consuming and the full immunohistochemical evaluation is often first known post-operatively. This study was designed to evaluate the accuracy of pre-operatively ultrasound guided fine needle aspirations (FNA's) for the detection of non-palpable lymph node metastases in primary breast cancer patients.

Material and Methods: We evaluated the material of 183 ultrasound guided FNA's of non-palpable axillary lymph nodes of primary breast cancer patients. The cytological results were compared with the final histological diagnosis and analyzed with descriptive statistical methods.