O-87 The role of CLEVER-1 in breast tumour metastasis

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Although the characterisation of lymphatic endothelial cells (LEC) has progressed little is known regarding the molecular events that regulate metastasis via lymphatics. We have been investigating the role of a novel lymphatic-associated adhesion molecule, CLEVER-1, in this process by assessing its expression in breast tumour specimens and by conducting in vitro experiments.

CLEVER-1 expression was examined in tonsil, lymph node (LN) and 66 breast carcinoma specimens by immunohistochemistry (paraffin-embedded sections). In vitro CLEVER-1 expression was also studied, via FACS, in HUVEC (human umbilical vein EC) and LEC (hTERT immortalised LEC) following exposure to a variety of stimuli (growth factors, cytokines and tumour conditioned media). Tumour cell and leukocyte adhesion to HUVEC and LEC were also examined.

Results show that, in tissue specimens, CLEVER-1 is present in blood and lymphatic capillaries and certain subpopulations of macrophage or dendritic cells. Although, in tumours, it is mainly expressed in blood vessels (62.1% vs 19.7% in lymph vessels) only lymphatic expression is significantly associated with LN metastasis ($p = 0.052$). Lymphovascular CLEVER-1 expression correlates with density of inflammatory infiltrate and expression in macrophage. In vitro results show that although CLEVER-1 is expressed intracellularly in both HUVEC and LEC only LEC exhibit surface expression. Interestingly, adhesion assays show that tumour cells adhere preferentially to LEC with maximal adhesion exhibited at 30–40 minutes. Such results are being further examined by using mAb blocking and siRNA knockdown along with overexpression studies to determine the role of CLEVER-1 in tumour cell adhesion and migration.

O-88 Survival of invasive breast cancer according to the Nottingham Prognostic Index in cases diagnosed in 1990 to 1999

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The Nottingham Prognostic Index (NPI) is a well established and widely used method of predicting survival of operable primary breast cancer. The index and survival figures were described in the 1982 and survival update of the original tumour set have been published with new cases added.

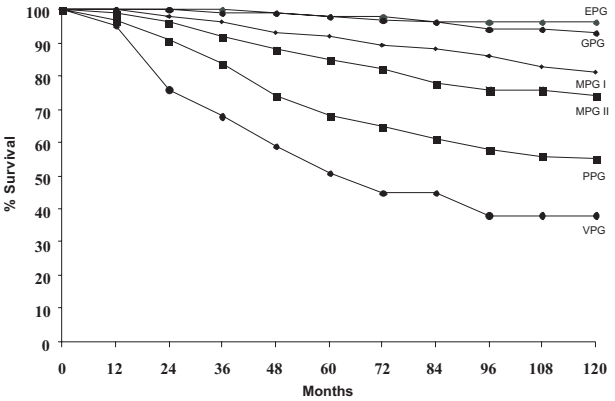
Survival in breast cancer has improved enormously and this paper presents survival figures for women diagnosed in the 1990's.

Aims: Primary: To present the survival figures for each NPI Group in women treated by up-to-date protocols. Secondary: From the observations to suggest reasons for the reported fall in mortality from breast cancer from that in the 1980's.

Methods: The NPI is compiled from grade, size and lymph node status of the primary tumour. Consecutive cases diagnosed and treated at Nottingham City Hospital in 1980–1986 ($n = 892$) and 1990–1999 ($n = 2238$) are compared. Changes in protocols towards earlier diagnosis and better case management were made in the late 1980's between the two data sets.

Results: Breast Cancer Specific Survivals are shown for each NPI group for cases diagnosed in the 1990's (Fig).

Case survival (Breast Cancer Specific) at 10 years has improved overall from 55% to 77%. Compared to cases treated in the 1980's, within all prognostic groups there are high relative and absolute risk reductions (a treatment effect). The distribution of cases to prognostic groups in the 1990's shows only a small increase from that in the early 1980's in the numbers in better groups (an early diagnosis effect).



Survival by NPI group (BCS): 1990–99.

Conclusion: Survival figures for women diagnosed in the 1990's are presented. Survival is greatly improved overall and in every prognostic group. The major part of the improvement in survival is due to improved case management with modern treatment protocols.

O-89 The effect of HER-2 overexpression on survival in early breast cancer

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Recent data suggest that there is a benefit to Her-2 testing for all patients with primary operable breast cancer as these patients may be suitable for trastuzumab therapy. Whilst there are recognized relationships between Her-2 positivity, poor prognosis and ER negativity, there is no data on the survival benefit in very early (ie Good or Excellent Prognosis) breast cancer.

Methods: Her-2 status was assessed using Herceptest and tissue microarray, in two archival consecutive datasets from the Nottingham Tenovus Primary Breast Cancer Series. The first was selected from patients (1980–1988) who received no adjuvant therapies ($n = 900$) and another from a series (1988–1995) treated with adjuvant systemic therapies ($n = 1900$).

Results: In both datasets there were no cases of HER-2 overexpression in patients in the Excellent and Good Nottingham Prognostic Index Groups. In the time period with no adjuvant therapy, the 10 year survival in the bottom three prognostic groups (ie MPG I, MPG II and PPG) was 40% vs 25% vs 14% respectively, for cases positive for HER-2 expression.

For those negative for Her-2, survival for the same period (by same prognostic groups) was 54% vs 31.6% vs 17.9%.

In those patients who received adjuvant chemotherapy, there was comparable reductions in survival even when adjusted for the influence of chemo and hormone therapy.

Conclusion: Her-2 overexpression is rarely if ever, present in patients who belong to the Excellent/Good Prognostic Groups (ie small, grade 1–2 tumours). Survival of in MPG I, II or PPG is significantly worse irrespective of the application of adjuvant therapies.
These data demonstrate that it is not useful or cost effective to apply testing to all cases of early breast cancer but rather those falling into poorer prognostic groups.
Also that chemotherapy does not correct the survival discrepancy between Her-2 positive and negative cases.

O-90 Prognostic significance of vascular endothelial cell growth factor (VEGF) -A, -C and -D in breast cancer and their relationship with angio- and lymphangiogenesis

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Vascular endothelial cell growth factors (VEGF)-A, VEGF-C and VEGF-D have potent angiogenic and lymphangiogenic functions in experimental models however their role in the progression of human breast cancer is unclear. The aims of the current study were to examine the relationship between the expression of the aforementioned VEGFs with the angiogenic and lymphangiogenic characteristics of breast cancer and to assess their suitability as potential prognostic factors.
Paraffin embedded sections of 177 primary invasive breast cancer, with complete clinical follow up information for 10 years, were stained for VEGF-A, -C, -D, podoplanin (to assess lymph vessel density (LVD)) and CD34 (to assess microvessel density (MVD)) using standard immunohistochemical approaches. The expression of the VEGFs was correlated with clinicopathological criteria, LVD, MVD and patients' survival.
High expression of VEGF-A, -C and -D was detected in 40%, 37% and 42% of specimens respectively. High expression of VEGF-A and VEGF-C, but not of VEGF-D, was associated with a higher LVD ($P = 0.013$ and $P = 0.014$ respectively), a higher MVD ($P < 0.001$ and $P = 0.002$ respectively), the presence of lymph node metastasis ($P < 0.001$ and $P < 0.001$ respectively), distant metastasis ($P = 0.010$ and $P = 0.008$ respectively) and shorter OS ($P = 0.029$ and $P = 0.028$ respectively).
In conclusion, breast cancers that express high levels of VEGF-A and VEGF-C are characterized by a poor prognosis, likely though the induction of angiogenesis and lymphangiogenesis. Examination of expression of VEGF-A and VEGF-C in breast cancer may help to identify a subset of tumours that have a higher probability of recurrence and metastatic spread.

O-91 Prognostic estimation: re-analysis of data from cases diagnosed in 1990–99 by Cox proportional hazards method

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The original Nottingham Prognostic Index (NPI) was based on a multivariate analysis by the Cox proportional hazards method, of 9 potential prognostic factors and described in 1982. Based on the 3 factors retaining independent significance the NPI is Grade (I-III) + LN Stage (1–3) + maximum diameter (cm $\times 0.2$).
The survival figures have been re-examined for each prognostic group for cases diagnosed in the 1990's (presented at this meeting) and the NPI still separate to six groups with significantly differing survivals, with wide separation between the best and worst groups.
The new analysis now identifies 5 factors showing independent significance and their relative contribution to h azard: the structure of the formula is preserved but a revised index may be calculated as: LN status (LN neg scores 1, LN neg LVI+ 1.5, LN 1–3 scores 2, LN 4+ scores 3) + Grade (I scores 0.7, II 2, III 3, III basal 3.4) + Size (cm x 0.2) + 0.6 for HER2neu positivity.
Using the revised index 20% of women have a change in their prognostic group (ie) a difference in their survival estimate of between 4 and 15%. However the change loses the simplicity of the NPI and for most women does not make a sufficient difference to alter treatment decisions.

O-92 UPARAP/ENDO180 expression in invasive breast carcinoma and its relation to patient outcome

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Introduction: Local growth, invasion, and metastasis of malignancies of invasive breast carcinoma involve extensive degradation and remodelling of the surrounding, collagen-rich connective tissue. Urokinase plasminogen activator receptor-associated protein (uPARAP)/Endo180 is an endocytic receptor recently shown to play a critical role in the uptake and intracellular degradation of collagen by mesenchymal cells. However, the expression of this protein and its clinical significance in breast cancer is unknown.
Methods: immunohistochemistry was used to investigate the expression of (uPARAP)/Endo180 in tissue microarrays of a large (n = 880) well-characterized series of human breast carcinomas using blinded semiquantitative scoring, in addition to a set of well known biological markers in breast cancer.
Results: (uPARAP)/was expressed in (5.7%) of invasive breast cancer, and in (78.8%) in the stroma surrounding these tumours. Positive expression of Endo 180 in the tumour cells was significantly correlated with negative steroid receptor as, ER (P = 0.013), and AR (P = 0.001), negative luminal cytokeratines like CK7/8(P = 0.041). further more, a positive correlation was found between Endo180 expression and basal subtype of breast carcinoma (P = 0.003). In addition to the association between its expression and shorter disease free interval (P = 0.01).
Conclusion: The association between (uPARAP)/Endo180 expression in malignant cancer cells with basal phenotype and its association with poor patient outcome could explain the aggressive behaviour of these types of tumour.

O-93 ALCAM is an independent predictor of survival in unselected breast cancer

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Introduction: Breast cancer is a heterogeneous disease and their complexity is demonstrated by the anomalies seen in their classification using histological-based criteria. There is a need to develop a molecular classification system to predict tumour behaviour, thus providing information on patient outcome and therapeutic response. To achieve this, key molecules associated with cancer biology need to be assessed as putative biomarkers.
Aim: To assess Activated Leukocyte Cell Adhesion Molecule (ALCAM) as a prognostic indicator of survival in breast cancer.
Materials and Methods: Tissue microarray (TMA) sections containing 196 well-characterised unselected breast tumours were immunohistochemically stained to detect