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P001-A: Short-term pregnancy hormone treatment on *N*-Methyl-*N*-nitrosourea-induced mammary carcinogenesis in relation to serum fatty acid composition

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Short-term estrogen and progesterone treatment (STEPT) mimics the pregnancy hormone milieu. This study compared the development of *N*-methyl-*N*-nitrosourea (MNU)-induced mammary cancer in female Lewis rats that received STEPT in early or later life. Rats in Groups 1 and 2 received a single intraperitoneal injection of 50 mg/kg MNU at 4 weeks old. Pellets containing 0.5 mg 17β-estradiol and 32.5 mg progesterone (EP) were subcutaneously implanted in rats in Group 1 during 6–9 weeks old. Rats in Groups 3 and 4 received 50 mg/kg MNU at 22 weeks old and again at 23 weeks old.

EP pellets were implanted in rats in Group 3 during 24–27 weeks old. At the time of EP removal and 8 weeks afterward, 4 randomly selected rats in each group were sacrificed for blood sampling. The fatty acid composition of serum phospholipids was measured by capillary gas chromatography. The remaining rats were sacrificed when they developed mammary tumours $\geq$1 cm in diameter or at the termination of the experiment, which was at 18 weeks old for Groups 1 and 2 and at 64 weeks old for Groups 3 and 4. Mammary cancer was histologically confirmed. Group 1 had a significantly suppressed incidence of mammary cancer compared to Group 2 (7% vs. 90%), whereas the cancer incidence in Group 3 was similar to that of Group 4 (50% vs. 56%).

Rats in Group 1 had significantly smaller n-6/n-3 polyunsaturated fatty acid (PUFA) ratios and higher levels of docosahexaenoic acid (DHA) than those in Group 2 at the time of EP removal but not 8 weeks after EP removal. Neither the PUFA ratios nor the DHA levels differed between Groups 3 and 4 at any time. These data suggest that the age at which STEPT is administered is important, since its mammary cancer-suppressing potential was lost in aged animals. DHA and the n-6/n-3 PUFA ratio may play a crucial role in mammary cancer suppression by STEPT.

P002-B: Identification of mammary gland development genes in mouse by ENU mutagenesis

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Rationale: The use of *N*-ethyl-*N*-nitrosourea (ENU) to induce mutations in mice has been a successful approach in identifying new gene functions, by correlating phenotypic effects with the induced genetic alterations. We were interested in using this approach to identify genes that regulate mammary gland development and lactation. Since genes that regulate normal mammary development are often associated with breast tumorigenesis, this project may provide new therapeutic targets for breast cancer.

Aims: To identify genes that regulates mammary gland development and lactation.

Methods: Lactational behaviour of mice derived from ENU-treated mice was determined by monitoring pup weight, milk spot and survival. Mammary glands were examined by whole mount and H&E stainings. Gene expressions and milk composition were determined by quantitative RT–PCR, immunohistochemistry and Western blotting. Cell proliferation and apoptosis were assessed by BrdU and cleaved-Caspase 3 stainings, respectively. Gene mutations were identified using PCR genotyping of polymorphic markers, followed by Next Generation sequencing. The expression pattern of the identified gene during mammary gland development was examined by both quantitative RT–PCR and immunohistochemistry.

Results: We identified a line of mice (Jersey) exhibiting inheritable dominant lactation failure. Ductal branching during pregnancy was normal. Alveolar development was reduced during late pregnancy and lactation. Mammary epithelial cell proliferation in early pregnancy is significantly less in Jersey glands, and is accompanied by increased apoptosis post-partum. Although milk was present in the Jersey glands, the alveolar lumens do not expand like the wild-type lactating glands, suggesting a reduction in milk volume. In addition, expression of beta-casein and whey acid protein in the milk are also decreased.

Two closely linked ENU-induced mutations were found in the affected Jersey mice. One was non-coding, and mice carrying null mutation of this gene showed normal lactation. The second mutation caused an amino acid change in a functional domain of the protein. The corresponding wild-type gene was found to be expressed in mammary epithelial cells at different stages development. We are currently investigating the potential mechanism of this mutation that results in the lactation phenotype.

Conclusion: We had identified a gene mutation which affects the proliferation, differentiation and apoptosis of the mammary epithelium at different stages of development.

P003-A: Identification of PUMA as an oestrogen target gene that mediates the apoptotic response to Tamoxifen in human breast cancer cells and predicts patient outcome in breast cancer

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Recognition of the pivotal role of oestrogen in the aetiology of breast cancer has led to the development of antioestrogens (AE) such as tamoxifen (TAM) as effective therapies for the treatment and prevention of this disease. However, despite their widespread clinical efficacy, response to AEs is often short-lived, and acquired or innate therapeutic resistance remains a major obstacle in the successful treatment of breast cancer. Thus, delineating the intracellular pathways that mediate the cellular response to oestrogen could potentially lead to new, more effective approaches to the treatment of breast cancer, particularly endocrine-resistant disease.

We have identified the BCL-2 homology 3 (BH3)-only, pro-apoptotic regulator, PUMA (p53 upregulated modulator of apoptosis) as an oestrogen target gene that is acutely downregulated (within 4 h) in response to estradiol (10 nM) in the human breast cancer cell lines, MCF-7 and T-47D. Although PUMA has an established role as a p53-upregulated gene and essential mediator of p53-dependent apoptosis, oestrogen's regulation of PUMA occurs independently of the p53 status of the cells. Furthermore, maintenance of this transcriptional effect in the presence of cycloheximide supports the concept that PUMA is a primary target of ERα in breast cancer cells.

PUMA is transcriptionally upregulated in T-47D and MCF-7 cells following treatment with TAM (7.5 μM; within 8 h), and knock down of PUMA expression in these cells with specific siRNA significantly attenuates the apoptotic response to TAM compared to non-targeting siRNA controls ($p < 0.05$). A publically available gene expression data set was analysed to identify relationships between PUMA mRNA expression and breast cancer patient outcome. PUMA protein expression was assessed using immunohistochemistry in 292 patients with invasive ductal carcinoma, and correlated with clinicopathological variables and disease outcome. Results showed that low PUMA mRNA and protein expression in breast carcinomas was significantly associated with breast cancer-specific death ($P = 0.0014$ and $P = 0.0115$, respectively).

These findings suggest that the dysregulation of apoptotic signaling pathways such as those executed via PUMA, can significantly impact on both the progression and therapeutic responsiveness of breast cancer. Moreover, they provide a convincing rationale for exploring new therapeutic approaches involving endocrine and non-endocrine therapies that target apoptotic pathways as an effective strategy for tackling endocrine refractory disease.

P004-B: Progesterone and Elf5 in mammary gland development

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Elf5 is a key transcription factor that specifies alveolar cell fate in the mammary gland during pregnancy [1]. The ability of Elf5 to rescue the failed development of prolactin (PRL) receptor null mammary glands has established a major role for Elf5 in mediating PRL action during pregnancy [2]. We have recently reported that Elf5 is also regulated by progesterone (P) in cell lines and mice [3], raising the possibility that Elf5 co-ordinates both P and PRL actions. The aims of this study were to determine whether Elf5 mediates P action, and to investigate how P and Elf5 co-operate during mammary development.

Elf5 transgenic mice were crossed with PRLacZKI animals to allow induction of Elf5 in mammary glands null for PR, and transplantation studies were used to assess the development of these glands during pregnancy. To study the combined effects of Elf5 and P, Elf5 transgenic mice were implanted with a sub-cutaneous slow-release P pellet and Elf5 expression was induced by doxycycline administration.

Induction of Elf5 did not rescue the failed development of PR null transplants, indicating that Elf5 is not primarily responsible for P action during pregnancy. However, Elf5 and P had additive effects on endpoints of mammary gland differentiation that may be reflected at the level of luminal progenitor cells. Most notably, in the presence of P, Elf5 promoted the formation of clusters of polarised alveolar buds. This phenotype resembles that achieved by over-expression of RANKL [4], a mediator of P's paracrine actions. We also report that PR and Elf5 are expressed in neighbouring mammary cells, consistent with the idea that P induces Elf5 expression via a paracrine mechanism. In conclusion, Elf5 co-operates with P to promote alveolar bud formation. Further work will investigate whether RANKL mediates P induction of Elf5 expression.

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P005-A: Defining the role of the ETS transcription factor Elf5 in the breast cancer phenotype

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Elf5 (e74-like factor 5) is a member of the ETS family of transcription factors. This family is involved in the regulation of cellular differentiation, development, cell cycle control, proliferation, survival and tumorigenesis. Elf5 mediates prolactin action; high Elf5 expression is essential for lactation, specifying differentiation of the CD61+ mammary progenitor cell population to form the secretory cell lineage. Comparison of gene expression profiles between patient-matched breast tumour and normal samples revealed that Elf5 is one of the most consistently down-regulated genes during carcinogenesis, with a large loss of Elf5 expression observed in luminal cancers and more moderate loss by the basal subtype. Thus higher Elf5 expression is associated with high-grade tumours, lymphocytic infiltration and with BRCA1 mutation, reflecting the basal-luminal differences in these features. The recent identification of the CD61+ progenitor cell as the source of BRCA1 tumours, together with higher Elf5 expression in Basal cancers, suggest the CD61+ progenitor cell as the origin of basal breast cancers and that Elf5 may be a key driver of the basal phenotype.

In the present study, we have investigated the effect of Elf5 on breast cancer cell phenotype and in mouse models of carcinogenesis. For that purpose, we have developed a doxycycline (Dox) inducible model of Elf5 expression in basal and luminal human breast cancer cells. Forced expression of Elf5 to levels seen in normal epithelium inhibited cell proliferation both in vitro and in vivo, via mechanisms involving cell cycle and survival parameters. Elf5 over-expressing cells displayed a reduction in the number of colonies in soft agar, indicating reduced anchorage-independent growth. In vivo studies reveal that Elf5 over-expression prevented tumour growth in subcutaneous xenografts of breast cancer cell lines in athymic nude mice. Using the intraductal cell xenotransplantation model, which provides a more appropriate niche for the tumour cells, we observed a reduction in tumour formation by the Elf5 over-expressing breast cancer cells. Furthermore, using the polyoma middle T oncoprotein (PyMT) mouse model, we have created a triple transgenic mouse carrying PyMT and a Tet-On MTB-promoter Elf5 construction. Although overall tumor incidence and growth rate are similar in Elf5 expressing mice compared to controls, Elf5 expression shows heterogeneous patterns of intratumoral expression and immuno-fluorescent studies using BrDU reveal a very marked reduction in the proliferation in areas of the tumour with high Elf5 expression. Efficient carcinogenesis requires reduced expression of the Elf5 transgene.

Thus forced re-expression of Elf5 to levels seen in normal breast epithelium stops luminal and basal breast cancer cell proliferation both in vitro and in vivo as well as the tumour formation of xenografts in immuno-compromised mice. This provides the first study of the in vivo effect of Elf5 over-expression in breast carcinogenesis and indicates that therapeutic strategies designed to mimic the effects of Elf5 re expression may prove efficacious for the treatment of breast cancer.

P006-B: Proteomics of invasive and metastatic human breast carcinoma

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Introduction: Metastasis includes loss of cellular adhesion, invasion into extracellular matrix, entry into circulation, exit into new tissue and colonization of a distant site. The primary objective of this study is to establish an isogenic model of invasiveness and study mechanism of cancer invasion by proteomics and systems biology approaches.

Experimental Procedures: By selecting the cells invaded into gelatin, highly invasive clones of MCF-7 cells were obtained for further study. 2D gels of parental MCF-7 cells, invasive MCF-7c46 cells and invasive, metastatic MDA-MB-231 cells were generated and differently expressed proteins were identified by mass spectrometry.

Systemic analyses were performed, included functional clustering, pathway and network analysis. Networks formed by identified invasiveness-related proteins were used for selection of proteins for further study.

Selected proteins were validated by immunoblotting with specific antibodies. To manipulate levels of selected proteins, overexpression and siRNA knockdown experiments were performed, and cell invasiveness, proliferation and apoptosis were measured.

Summary of data. We isolated a highly invasive clone of MCF-7 cells, MCF-7c46. Invasion assay showed that their invasiveness across matrigel barrier is 10-fold higher than their parental cells. The subsets of differentially expressed proteins from 2D gel were defined to extract invasiveness-specific proteins, i.e. MCF-7 v.s. MCF7c46 (non-invasive v.s. invasive), MCF-7 v.s. MDA-MB-231 (non-invasive v.s. invasive, metastatic), and MCF-7c46 v.s. MDA-MB-231 (invasive v.s. invasive, metastatic). By functionally analysis, all three subsets of proteome profile showed significant similarity in affected functional domains, but ECM organization and biogenesis were more represented in non-invasive v.s. invasive subset, i.e. MCF-7 v.s. MCF7/46, and MCF-7 v.s. MDA-MB-231. Systemic analysis of invasive-specific network, which is a overlapped network of the subsets MCF-7 v.s. MCF7c46, and MCF-7 v.s. MDA-MB-231, revealed several major hubs, e.g. TGFbeta, EGF, VEGF, IGF, NFkabbaB and HNF. The network analysis showed relations between these regulatory processes.

Subsequently, the key regulators for breast carcinoma invasion were selected for validation and functional and study.

Conclusions: New isogenic model of invasiveness of human breast epithelial cells has been generated. Major hubs in the invasiveness-specific network have been identified. They include TGFbeta, EGF, VEGF, IGF, NFkabbaB and HNF signaling mechanisms in defined relations, e.g. these mechanisms are involved in regulation of invasiveness in a defined order and hierarchy. Network analysis suggested that BRMS1 is a key regulator of breast carcinoma invasiveness and its regulatory mechanism is currently under investigation.

P007-A: Tamoxifen-induced epigenetic silencing of oestrogen-regulated tumour suppressor genes as a novel concept in anti-hormone resistant breast cancer

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Previous studies at the Tenovus Centre have demonstrated that the development of tamoxifen-resistance in vitro can be attributed to aberrant growth-factor receptor signalling, which facilitates advanced

aggressive behaviours in breast cancer cells such as increased proliferation. The aim of the present study was to determine how these undesirable features of resistant cells were affected following withdrawal from tamoxifen.

Interestingly, in MCF-7-derived tamoxifen-resistant cell sub-lines that had been withdrawn from tamoxifen for up to 6 months, the increased rate of proliferation was sustained despite the reduced expression of epidermal growth factor receptor (EGFR) and reduced sensitivity to EGFR signalling inhibition. Here, evidence is presented to suggest that the accelerated rate of proliferation was in part maintained by epigenetically modified oestrogen responsive tumour suppressor genes, silenced following long-term tamoxifen exposure.

Using quantitative PCR, immunocytochemistry and MethyLight technology, proof-of-principle studies demonstrated the methylated status of promoter sequences for the prototypic oestrogen-responsive genes, trefoil factor-1 (pS2) and progesterone receptor in our resistant cell model. The 'silent state' of these promoters was reversed using a combination of the de-methylation agent 5-Azacytidine (5-Aza) and oestradiol (E2). Surprisingly, 5-Aza treatment enabled E2, a modest mitogen in the absence of this agent, to become a significant inhibitor of resistant cell growth. This was apparent over physiological levels of E2 (1×10^{-9} M–1×10^{-7} M), with the maximal dose of E2 causing a 50% reduction in the number of cells following 7 days culture compared to non-E2 (+5-Aza) treated cells ($p < 0.001$). In contrast, cells cultured in the absence of 5-Aza showed a 40% increase in cell number with E2 at 1×10^{-7} M (compared to non-E2/non-5-Aza treated control cells) ($p < 0.001$). Further analysis showed that the addition of tamoxifen (1×10^{-7} M) to cells cultured with 5-Aza and E2 reversed this growth suppression; hence it could be argued that the co-addition of tamoxifen suppressed the genes reactivated by 5-Aza/E2 co-treatment that are responsible for this phenomenon.

Subsequent microarray analysis revealed a cohort of genes whose ontology indicated tumour suppressor/pro-apoptotic function, including Growth Differentiation Factor-15 (a TGFβ-superfamily member reported to lie down-stream of p53), as being methylated during long-term tamoxifen exposure. Such genes were re-expressed when cells were treated with 5-Aza + E2 in parallel with growth inhibitory effects.

Thus, the data in this study provides evidence to support a novel concept that directly links anti-hormone-induced hypermethylation of oestrogen-regulated tumour suppressor gene promoters to acquired anti-hormone resistant cell growth, and highlights a previously unrecognised therapeutic opportunity in tamoxifen-resistant breast cancer. The identification of genes associated with this phenomenon could provide further insight into the mechanisms that contribute to acquired tamoxifen-resistant cell growth, while their profiling could also potentially have significant predictive response value. Furthermore, the restoration of their expression or function on an individual basis may ultimately be valuable in improving treatment of tamoxifen resistant breast cancer and may be more desirable than epigenetic manipulation, which may have a broader genomic impact in cancer.

P008-B: Pathology and clinical impact of Chromosome 17q12-q21.2 genes copy number amplification in HER2 positive breast cancers

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It has been described that amplifications in the chromosome 17 (ch17) could affect different genes implicated in cancer development. The aim of the study was to determine, by quantitative PCR (qPCR), the amplification level of different genes located on ch17q12-21in HER2 positive breast cancers.

We determined GRB7, TOP2A, THRA, IGFBP4, CCR7, KRT20, KRT19 and GAS gene copy numbers by quantitative PCR in 86 HER2 amplified and 40 HER2 negative breast tumors. Patients were included between 2002 and 2006 (median follow-up = 55 months). They received radiotherapy (100%), anthracycline based regimen (79%), Herceptin (23%) and hormonotherapy (40%) as adjuvant therapy according to therapeutic standard used at the time of surgery. qPCR reactions were performed on a Rotorgene 6000 (Qiagen) using Absolute Blue QPCR SYBR Green Mix (ref AB-4167, ThermoFisher Scientific). Amplification levels were normalized with the somatostatin receptor type II (SSTR2) gene Quantification of SSTR2 was also normalized with GAPDH and ACBT in order to detect SSTR2 amplification. The quantification was achieved by constructing a standard curve from serial dilutions of normal genomic DNA (ref 11 691 112 001, Roche, France). Gene amplification was defined by a ration target gene/reference > 2.

We confirm that GRB7, TOP2A, THRA, IGFBP4, CCR7, KRT20, KRT19 and GAS amplification occurs only in patients with HER2-amplified breast tumor. Gene amplification occurs in 100% for GRB7, 55% for THRA, 23% for TOP2A, 19.5% for IGFBP4, 18.4% for CCR7, 13.8% for KRT20, 11.5% for KRT19, and 6.9% for GAS. We show that HER2 presents the highest value of amplification and that amplification frequency of a gene decrease with the distance from HER2. The amplification level of HER2 is not correlated with TOP2A amplification's levels (rho = 0.11) nor with other genes amplification levels. Gene amplifications are not correlated with T and SBR. TOP2A, IGFBP4, KRT20 and KRT19 amplification are statistically significantly associated with node positive breast cancers ($p = 0.077$, 0.072, 0.001 and 0.019 respectively).

The HER 2 amplicon do not include GRB7, TOP2A, THRA, IGFBP4, CCR7, KRT19, KRT20 and GAS. It leads to increased gene copy number alterations in Chr17q 12-21 that could be related with an invasive breast cancer profile.

P009-A: Functional significance of TRIM22 in breast cancer

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Tripartite motif-containing 22 (TRIM22) is a member of the TRIM protein family, characterized by the presence of RBCC motif. It is inducible by both type I and type II interferons (IFNs) and possesses antiviral activity against viruses such as Human Immunodeficiency Virus. It has been identified to be an E3 ubiquitin ligase and a down-stream target gene of p53. To characterize the function of TRIM22, we studied the response of TRIM22 to apoptosis inducing drugs. We showed that TRIM22 expression is induced during cell cycle arrest in a p53-dependent manner. In addition, we have demonstrated that

TRIM22 nuclear level increased drastically after IFN-β and mitomycin C treatment, suggesting that nucleus is the possible site of action for TRIM22. Western blotting analysis performed on a panel of mammary epithelial cell lines (3 non-malignant mammary epithelial cell lines and 11 breast cancer cell lines) revealed that TRIM22 expression is high in non-malignant mammary epithelial cells but hugely downregulated in malignant breast cancer cell lines. Similar result was obtained when we examined the TRIM22 protein expression in 63 pairs of human breast tumor tissue and the adjacent normal tissue. We found the level of TRIM22 protein is higher in the adjacent normal tissue than the tumor tissue. These results suggest possible growth inhibitory role of TRIM22 and its association with breast cancer development.

P010-B: Generation and characterization of Tetratricopeptide repeat domain 9A knockout mice

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Tetratricopeptide repeat domain 9A (TTC9A) was originally cloned from a cDNA library of the brain. It was later found to be drastically induced by progesterone in breast cancer cells and this induction is associated with growth-inhibitory effect of progesterone. TTC9A was also found to be significantly over-expressed in breast cancer tissues as compared to the adjacent normal tissues. However, information on its regulation of expression is limited and its function is not clear. To investigate further the function of TTC9A in mammary development and mammary carcinogenesis, we have generated mice with deletion of TTC9A gene using gene targeting to replace exon 1 with neomycin cassette. Despite the dynamic expression of TTC9A in the developing mammary gland, nervous system, and other organs, TTC9A-deficient mice are viable, fertile, and appear to be normal. This report describes the characterization of mammary and brain development in TTC9A knockout mice that will shed light to the role of this gene in development.

P011-A: Splice variant profiling of tamoxifen sensitive and resistance breast cancer

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Tamoxifen is a selective estrogen receptor modulator (SERM) widely used to treat estrogen receptor (ER) positive breast cancers. However many patients eventually acquire resistance and relapse. Gene expression profiling studies have identified gene profiles that could better classify cancer subtypes, predict cancer prognosis or characterize tamoxifen responsiveness, but with little concordance between the genes identified. This may partly be due to pre-mRNA alternatively spliced variants that are not discriminated in conventional microarrays. The purpose of this study was to identify alternatively spliced variants that contribute to tamoxifen resistance in breast cancer.

SpliceArray^{TM} profiling (ExonHit Therapeutics, Inc.) of 417 breast cancer-related genes was performed in a panel of breast cancer cell lines. Splice variants that were differentially expressed between parental tamoxifen-sensitive (TamS) and derived tamoxifen-resistant (TamR) cell lines were identified and validated by real time-quantitative PCR.

Splice variant BQ323636.1 of NCOR2 (Nuclear receptor co-repressor 2) was successfully validated in cell lines and clinical samples. In presence of tamoxifen, the mRNA expression level ratio of variant (BQ323636.1) versus wild type form (NM_006312.2) was significantly higher in derived TamR cell line AK47 compared to its parental TamS cell line ZR75-1. In 25 Chinese breast cancer patient RNA samples, the ratio positively correlated with metastasis.

NCOR2 is a component of the histone deacetylase-containing protein complex. It is recruited by tamoxifen to repress the transcription activation activity of ERα. BQ323636.1 variant has exon 11 skipped, resulting in early termination of the protein product. Only the first repression domain at the N-terminal is retained while 3 repression domains and 2 nuclear receptor-interacting domains are lost, indicating its disability of binding to nuclear receptors. Thus, this variant may hinder the repression on ERα transcription activation activity by competing with its wild type in binding with other protein partners in the HDAC complex, hindering its recruitment to the target gene promoter and modulating the antagonist effect of tamoxifen. Functional studies are being performed to confirm this possible mechanism, which may serve as a potential therapeutic target to overcome acquired tamoxifen resistance in breast cancer.

P012-B: Cyclin E2 is expressed in the S phase of cancer cells and increases genomic instability

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Cyclin E1 is dysregulated in cancer and associated with poor patient outcome, supporting a causative role in tumorigenesis. High expression of cyclin E1 increases proliferation, while dysregulation of periodic expression increases genomic instability. Cyclin E1 is regulated via periodic expression and degradation: expression peaks at the G1/S phase transition, and it is then rapidly degraded during S phase after recognition by the SCF(Fbw7) ubiquitin ligase. The closely related protein, cyclin E2, has been presumed to be redundant with cyclin E1. We show that cyclin E1 and E2 are expressed in distinct subpopulations of cancer cells, due to a failure to co-ordinately degrade the two proteins. Cyclin E2 protein expression was maintained during S phase while cyclin E1 expression decreased over the same period. This is likely due to failed targeting of cyclin E2 by Fbw7, as the inactivation or depletion of Fbw7 led only to significant changes in the stability of cyclin E1, but not cyclin E2. Cyclin E1 and E2 were concordantly expressed and regulated in normal cell lines, suggesting that cyclin E2 regulation is specifically perturbed in cancer cells.

Expression of cyclin E1 during S phase leads to genomic instability. Since we observed S phase expression of cyclin E2, we assayed the effect of overexpression of cyclin E1 and E2 on the induction of micronuclei, a marker of genomic instability. Cyclin E2 induced micronucleation at a greater rate than cyclin E1. These data imply that cyclin E2 may be a stronger promoter of genomic instability due to its expression patterns. Overall, we have identified key differences between cyclin E1 and E2 in cancer cells that indicate that they are independently regulated and may be functionally distinct.

P013-A: A genome-wide association scan on ER-negative breast cancer

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Introduction: Breast cancer is a heterogeneous disease and may be characterized on the basis of whether estrogen receptors (ER) are expressed in the tumour cells. ER status of breast cancer is important clinically, and is used both as a prognostic indicator and treatment predictor. In this study, we focused on identifying genetic markers associated with ER-negative breast cancer risk.

Methods: We conducted a genome-wide association analysis of 285,984 SNPs genotyped in 617 ER-negative cases and 4,583 controls. We also conducted a genome-wide pathway analysis on the discovery dataset using permutation-based tests on pre-defined pathways. The extent of shared polygenic variation between ER-negative and ER-positive breast cancers was assessed by relating risk scores, derived using ER-positive breast cancer samples, to disease state in independent, ER-negative breast cancer cases.

Results: Association with ER-negative breast cancer was not validated for any of the 5 most strongly associated SNPs followed up in independent studies. However, an excess of small *P*-values for SNPs with known regulatory functions in cancer-related pathways was found (global $P = 0.052$). We found no evidence to suggest that ER-negative breast cancer shares a polygenic basis to disease with ER-positive breast cancer.

Conclusion: ER-negative breast cancer is a distinct breast cancer subtype that merits independent analyses. Given the clinical importance of this phenotype and the likelihood that genetic effect sizes are small, greater sample sizes and further studies are required to understand the etiology of ER-negative breast cancers.

P014-B: Identification of genes collaborating with Her2/ErbB2 in mouse mammary tumor models for human breast cancer

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MMTV proviral insertion in the genome of murine mammary epithelial cells can activate flanking proto-oncogenes leading to mammary tumor induction. We performed an insertional mutagenesis screen in mammary tumors from MMTV-ErbB2 transgenic mice. We identified Eras, Irs4 and Igf2 as frequent MMTV targets in these mice but rarely genes acting in the Wnt and Fgf pathways. In contrast, in wild-type mice the latter genes were the most frequent MMTV targets, whereas the former genes were only occasionally tagged. Among the three former genes overexpression of Eras was most significantly associated with ErbB2 positive tumors. ERAS is a constitutive active Ras-like GTPase, normally only expressed in embryonic stem (ES) cells. It has been reported that ERAS in ES cells only activates the PI3-kinase but not the MAP-kinase pathway. Overexpression of both Eras and Irs4 in immortalized normal mammary epithelial cells (NMuMG cells) strongly increased the growth rate in vitro, induced anchorage independent growth and rendered these cells tumorigenic in nude mice, validating the mammary tumor inducing capacity of both genes. Moreover, each of these genes accelerated ErbB2 induced tumorigenesis when co-expressed in NMuMG cells. Eras and Irs4 strongly activated the PI3-kinase pathway in various mammary epithelial cell lines. It has been shown that ERBB2 in human and mouse mammary tumors forms a heterodimer with ERBB3. In this complex, ERBB2 mainly activates the MAPK pathway, while ERBB3 activates the PI3 K pathway. Since ErbB2 driven mammary tumorigenesis is highly dependent on PI3-kinase activity, we hypothesize that genes like Eras and Irs4 are targeted by MMTV in ErbB2 tumors that lack ErbB3 mediated PI3 K activation. Indeed, ErbB3 is highly expressed in all tumors that arise in non-transfected ErbB2 transgenic mice but only in 60% of the MMTV infected ErbB2 transgenic mice. Thus activation of the PI3 K-pathway via Eras, Irs4 and IgF2 can act as an alternative for the activation of this pathway through ERBB3, PTEN inactivation or PIK3CA mutations. Eras mRNA was also expressed to a variable degree in a small percentage of human breast cancers suggesting a possible involvement of this normally silent gene in human breast carcinogenesis.

P015-A: Silencing of the PP2A catalytic subunit causes reduced in PTPase activity that is mediated via PTP1B in HER-2/neu positive breast cancer cell lines

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The HER-2/neu proto-oncogene has been found to be overexpressed in 20–30% of breast cancer tumours. This ligandless receptor is able

to heterodimerize with members of the receptor tyrosine kinase family and causes autophosphorylation, whereupon signal transduction events are triggered. Thus, in cancer cells numerous heterodimers are formed, resulting in unabated aggressive tumour growth and death. However, relatively little is known of the cellular phosphatase(s) responsible for the activation/deactivation of the HER-2/neu signalling cascade. Protein tyrosine phosphatase 1B (PTP1B) is required for HER-2/neu transformation in human breast epithelial cells and inhibition of PTP1B delays HER-2/neu-induced mammary tumorigenesis. Our group had previously demonstrated that silencing protein phosphates 2A catalytic subunit (PP2A/C) causes the HER-2/neu positive breast cancer cells to undergo apoptosis and the PTPase activity was significantly reduced. Given that PTP1B is required for HER-2/neu transformation and its association with PP2A is critical for PP2A activation, we postulated that the decrease in tyrosine phosphatase activity is contributed by PTP1B. We further silenced the BT474 and SKBR3 HER-2/neu positive breast cancer cell lines using siRNA targeting the PP2A/C and examined the effect of silencing PP2A/C on the expression of PTP1B by Western blotting. Our results showed that the expression of PTP1B was significantly reduced in both PP2A/C silenced BT474 and SKBR3 breast cancer cell lines. Our preliminary study further confirms that the attenuation of PTPase activity is mediated by PTP1B. However, the association of PTP1B-PP2A and HER-2/neu is still on-going and remains to be further elucidated. Our results suggested that silencing of the PP2A/C leads to reduced tyrosine phosphatase activity that could be mediated by PTP1B, and thus, contribute to the deceleration of the cell proliferation and survival rate in HER-2/neu breast cancer cells.

P016-B: Role for JMJD6 in breast cancer

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The pathogenesis of breast cancer involves a multitude of genetic alterations affecting the regulation of cell growth, survival, invasion and metastasis. To discover such novel oncogenes, we analyzed comprehensive gene expression profiles of breast tumors from several breast cancer patient cohorts for correlations between gene expression and distant metastasis-free survival. Jumonji Domain Containing 6 Protein (JMJD6), a histone arginine demethylase was one of the genes associated with poor survival in all cohorts. Immunohistochemical analysis of JMJD6 in tissue microarrays suggested that its high expression significantly correlated with grade and estrogen receptor negativity. In addition, its over-expression in multiple breast cancer cell lines led to increased proliferation, while in contrast, siRNA-mediated knock-down suppressed cell division. Microarray analysis of gene expression changes induced by siRNA-mediated knock-down of JMJD6 suggested that JMJD6 led to increased expression of cell cycle genes including CDC6, CHEK1, and suppression of cell cycle inhibitors like PTEN and TGF-ÃŽÂ[2]. We confirmed that siRNA-mediated knock-down of JMJD6 increased TGF-beta 2 secretion whereas JMJD6 over-expression reduced it. Since TGF-ÃŽÂ[2]s have been implicated in cell cycle arrest in many epithelial cells, we propose that JMJD6 may influence cell growth primarily via this pathway. Further studies to understand the molecular relationship between JMJD6 and TGF-ÃŽÂ[2] regulation are underway. These results suggest that the extrapolation of gene-survival associations in primary tumors to phenotypic analysis in vitro holds promise as a platform for understanding of new mechanisms in tumorigenesis, as well as discover new therapeutic targets.

P017-A: BRIP1 and PALB2 mutation detection in Hunter-New England familial breast cancer cohort

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Familial breast cancer accounts for 10% of breast cancer cases, and only 15-20% of these cases arise as a result of mutations in the breast cancer susceptibility genes, BRCA1 and BRCA2. This remainder of the cases have not been attributed to any other mutations, and studies to discover novel breast cancer susceptibility genes have been unsuccessful. BRIP1 and PALB2 are genes that encode for proteins that are involved in the BRCA1 DNA damage repair pathway. Mutations in these genes have been shown to confer an approximately two-fold increase in the risk of breast cancer compared to population risks in patients who have Fanconi anemia. However, the incidence of these mutations in hereditary breast cancer is unknown. The aim of this study was to determine if BRIP1 and PALB2 mutations are prevalent in hereditary breast cancer cases that do not have mutations in BRCA1 and BRCA2. The coding sequences of BRIP1 and PALB2 with exon/intron boundaries were analysed by direct sequencing methods on genomic DNA of 62 breast cancer patients that are negative for BRCA1 and BRCA2 mutations. Preliminary results showed single base changes in BRIP1: 2755T > C (S919P), 2637A > G (E879E), and PALB2: 2014G > C (E672Q), 3114-51T > A. The identification of these mutations may enable the translation of research output to clinical practice by offering genetic testing of BRIP1 and PALB2 to breast cancer patients referred for genetic screening.

P018-B: Combined genomic and phenotype screening to discover novel therapeutic targets in breast cancer

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Secretory factors and extracellular receptors that drive cancer progression make attractive immunotherapeutic targets. To discover such novel targetable oncogenes, we used a whole-genome data-mining approach that takes advantage of large independent microarray studies of breast tumors annotated for clinical outcomes. Genes without previous implication in breast cancer were identified based on their significant association with cancer recurrence in multiple cohorts, and further filtered for secretory factors and extracellular receptors based on gene ontology. A high-content screening strategy was then applied to the candidates to assess the phenotypic consequences of altering candidate gene expression. This screen suggested that serine protease inhibitor Kazal-type 1 (SPINK1), a secreted extracellular factor, was associated with breast cancer aggressiveness. We demonstrate that enforced SPINK1 expression and exposure to exogenous recombinant SPINK1 induce both invasiveness and survival of breast cancer cells and down-regulation of SPINK1 is associated with cancer cell death. Moreover, increased expression of SPINK1 resulted in an increased resistance to drug-

induced apoptosis. Immunohistochemical analysis of breast cancer tissue microarrays suggested that high SPINK1 expression was not seen in normal breast but is apparent in advanced disease. Moreover, SPINK1 shows nuclear localization primarily in higher grade breast cancers. This nuclear localization was recapitulated when tagged exogenous SPINK1 was applied to cells in vitro. SPINK1 is therefore a marker for cellular robustness in breast cancer and maybe a potential therapeutic target in this disease.

P019-A: Radiation induced and telomere associated genomic instability in breast cancer patients

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The study was an attempt to correlate the radiation-induced damage in the peripheral blood lymphocytes (PBLs) of breast cancer patients with the frequency of telomere mediated chromosomal damage. Blood samples from 55 patients with (Gr-II and Gr-III) CA-breast were obtained pre- and post-radiotherapy. The patients were treated with external beam radiotherapy of 50.4 Gy over a period of 6 weeks. Chromosome damage was measured by analyzing micronucleus (MN) frequency in PBLs. The MN-frequency of the irradiated patients increased significantly compared to the patients being self-controls. The micronuclei were hybridized with telomere probes to study the extent of telomere damage. The fluorescence signals of the telomere regions in the first generation of the bi nucleated cells were significantly higher in the post-radiotherapy patients.

There was also significant correlation observed in the patients with higher-grade tumors. Inter-individual variability was observed in the radiation-induced MN frequency in lymphocytes of patients after 6 weeks of radiotherapy. There was a significant correlation between functionally intact telomeres and the cellular response to ionizing radiation. Our findings suggest that fluorescence in situ hybridization on micronuclei could be effectively used as routine clinical application to determine the individual sensitivity to ionizing radiation with respect to telomere damage.

Sixty-one archived breast tissues (38 cancer tissues and 23 paired normal tissues) were used in another study. The breast tumor tissues showed significantly shorter telomeres (7.7 kb) compared with the paired adjacent tissues (9.0 kb) by Southern blot analysis. Moreover, telomere shortening was more significant in Grade III tumors than in the Grade II tumors (P 5 0.05). Quantitative fluorescence in situ hybridization on paraffin tissue sections revealed a similar trend in telomere shortening. Telomere attrition was associated with telomere dysfunction as revealed by the presence of significantly higher anaphase bridges in tumor cells which was tumor grade dependent. Furthermore, estrogen receptive negative tumors displayed higher anaphase and internuclear bridges. Selected samples from each grade showed greater genomic imbalances in the higher grades than the lower grade tumors as detected by array-comparative genomic hybridization.

Telomerase activity was found to be higher in the higher grades (Grade II and III) compared with the lower grade (Grade I). The average mRNA expression of TRF1 and POT1 was lower in the tumor tissues than in the normal tissues. Tankyrase 1 mRNA expression showed a grade-dependent increase in tumor tissues and its expression was also high in estrogen and progesterone negative tumors. The data support the notion that telomere dysfunction might be of value as a marker of aggressiveness of the tumors in breast cancer patients.

P020-B: Loss of Mel-18 enhances the breast cancer stem cell activity and tumorigenicity through activating the Notch signaling mediated by the Wnt/TCF pathway

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Mel-18 has been proposed as a tumor suppressor and negative regulator of Bmi-1, a cancer stem cell (CSC) marker. However, it is still unclear whether Mel-18 is involved in CSC regulation. Here, we examined the role of Mel-18 in the regulation of stemness of human breast CSCs. In Mel-18 shRNA-transduced MCF7 cells, side population (SP) cells and breast CSC surface marker (CD44 +/CD24-/ESA +) expressing cells were enriched. In these cells, the ability for tumorsphere formation and anchorage independent cell growth were accelerated, indicating the enhanced self-renewal of CSCs by Mel-18 knockdown. Mel-18 blockade by the shRNA also increased the breast tumor-initiating capacity in vivo. Using Stem cell PCR array, we found that the expression of Notch ligand Jagged-1, which is a stem cell specific marker and target of Wnt/TCF pathway, was upregulated by Mel-18 knockdown. Furthermore, the expression of Notch receptors Notch 1 and 3 were also increased, and nuclear accumulation of cleaved Notch intracellular domain (NICD) and its target Hes-1 transcription were consequently upregulated by Mel-18 knockdown. These effects were blocked by the treatment of β -catenin/TCF inhibitor or Jagged-1 siRNA, indicating Wnt/TCF pathway-mediated Notch activation by Mel-18 blockade. Pharmacologic inhibition of Notch and Wnt pathway abrogated the increased tumorsphere formation by Mel-18 downregulation. Taken together, our findings provide that Mel-18 is a novel negative regulator of breast CSCs that inhibits stem cell population and in vitro and in vivo self renewal through the inactivation of CSC self renewal pathway, Wnt and Notch signaling, suggesting the novel tumor suppressive role of Mel-18.

P021-A: Constitutive overexpression of Id-1 in mammary glands of transgenic mice results in precocious and increased formation of terminal end buds, enhanced alveologenesis, delayed involution

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Inhibitor of differentiation-1 (Id-1) has been shown to play an essential role in cell proliferation, invasion, migration and anti-apoptosis. However, the effect of Id-1 in mammary gland development remains unknown. Here, we generated MMTV-Id-1 transgenic mice to study the role of Id-1 in mammary gland development. In virgin mice, Id-1 overexpression led to precocious development and delayed regression of terminal end buds (TEBs) compared with wild type mice. The number of BrdU-positive cells and the expression of Wnt signaling molecules, β-catenin and cyclin D1, which regulate ductal extension and TEB formation in virgin, were statistically higher in Id-1 transgenic mice than in wild type mice. Id-1 also had an effect on the formation and proliferation of lobuloalveolar structures during early and midpregnancy. Id-1 transgenic mice had more lobulated and prominent alveolar budding than wild type mice and had significantly greater counts of lobuloalveolar structures in early pregnancy. The expression of BrdU, β-catenin and cyclin D1 was also predominantly increased in Id-1 transgenic mice. Moreover, Id-1 transgenic mice showed delayed involution. Id-1 regulated the expression levels of anti-apoptotic Bcl-2 and pro-apoptotic Bax, and resulted in delay of apoptotic peak during postlactational involution. We also found that Id-1 was able to modulate expression of the regulators of Wnt/β-catenin signaling such as phospho-Akt, BMP2, FGF3 and RAR-β in tubuloalveolar development of mammary glands. Taken together, our results suggestthat Id-1 plays a pivotal role in mammary gland development through Wnt signaling-mediated acceleration of precocity and alveologenesis and Bcl-2 family members-mediated delay of involution.

P022-B: Using expression profiling to identify recurrent amplicons

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Up regulation of transcriptomic dosage usually occurs with amplification of genomic loci. In breast cancer, it has been illustrated that gains of genomic loci result in coordinate expression of the respective genes. An assessment of this feature, utilizing the combination of genome wide microarray expression data and patient profiling of 4 different patient cohorts with aggressive breast tumors, we proposed that recurrent amplicons may exist on 17q12, chr8p11-12, chr11q13 and others. These regions were scored and ranked based on gene expression levels and frequency across the tumors. Genes that resided in these amplicons were analyzed for their correlation to poor patient outcome and were shortlisted as potential candidate oncogenes. In addition, each of these genes was further analyzed in a pair-wise manner to determine if co-expression of such pairs improved their prognostic value. Our results show that the top pair MCM10 and HOMER3 delivered a prognostic p-value of 6×10^{-13} which is $>6 \times 10^{4}$ times better than the prognostic values the individual genes. We further validated that the predicted chromosomal aberrations and co-expression of the gene pairs indeed existed in published primary tumor sequencing and array CGH data. Further analysis to determine the molecular pathways and mechanisms controlled by these pairs is underway.

P023-A: Weak genetic factor from Malaysian breast cancer patients

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Introduction: Breast cancer is a common malignancy affecting women globally. In Malaysia, it constitutes 31% of all newly diagnosed female cancer cases. Approximately 50% of the cases occur in women under the age of 50 years. A proportion of these cases may be attributable, at least in part, to genetic components. Women who carry BRCA1 mutations have a probability of about 80% for developing breast cancer, and 40 to 60% for developing ovarian cancer during their lifetime. In a previous study by the same authors mutations (detected by DHPLC using a different primer) were detected in 12.5% (5/40) of breast cancer patients. Three of them were Chinese, one Indian and one Malay. In this study the number of patients was increased to 100. Mutations were detected using PTT technique and then samples were sequenced. Aim of Study: 1. To investigate and analyze the role of genetic factor in breast cancer from Malaysian patients with either early-onset breast cancer (at age $\leq$ 45 years) or a positive family history. 2. To test the possible of setting up a screening and diagnostic test. 3. To explore the possible of development drug formulation for treating breast cancer. Materials & Methods: In collaboration with the IIUM Breast Centre and the Surgery Department at Hospital Tingku Ampuan Afzan (HTAA), Kuantan, 100 peripheral blood samples were collected from women with either early-onset breast cancer or a positive family history of breast cancer. Mutations were detected in exon 11 of BRCA1 gene by (Protein Truncation Test (PTT) followed by sequencing. Results: No truncated protein was detected in any of the 100 samples. Discussion and Conclusion: In spite of reports in literatures from other countries documenting evidence of protein truncation mutations in exon11 BRCA1 gene in early onset breast cancer patients, no such mutations where detected in this study. This may indicate a weak genetic factor involved in breast cancer from Malaysian patients, or we should look for mutations in other segments of exon11.

P024-B: Discovery of kinases associated with acquisition of triple negative phenotype during antihormonal treatment of ER+ breast cancer cells

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Introduction: Tumours which fail to express ER, PR and lack amplified HER2 ("triple negative" [TN]) comprise $\sim$15% breast cancer de novo with increased risk of early recurrence and poorer prognosis. As neither antihormones nor trastuzumab are applicable with only poor responses to EGFR inhibitors (despite $\sim$50% TN disease overexpressing EGFR), such TN tumours are under intense study to discover treatments, with promising findings for PARP inhibition. However, a TN state can also be acquired by ER+ breast cancers during antihormone therapy. 15–40% become ER- on antioestrogen relapse, some lacking HER2, while $\sim$10% ER + patients

were ER- (all HER2-) after neoadjuvant letrozole, associating with aggressive relapse and death. The signalling driving growth of tumours acquiring a TN state during antihormone treatment remains unknown. We hypothesise that modelling the acquired TN state and deciphering its pathways will discriminate future therapeutic targets. Our objectives were thus to develop experimental models of acquired TN breast cancer (encompassing EGFR + and EGFR- TN phenotypes) and to derive gene expression data to discriminate kinases elevated in this state. Experimental procedures: In vitro cloning, coupled with immunocytochemical and RT–PCR screening of ER, PR, HER2 and EGFR status, was used to isolate EGFR+ and EGFR- TN acquired resistant human breast cancer cell lines following continuous antioestrogen treatment (10^{-7} M faslodex) of ER + MCF7 cells. Using triplicate RNA preparations microarrayed with Affymetrix HGU133A2.0 GeneChips and median normalising and log-transforming resultant data, we compared gene expression for the full kinome (899 probes) between these models, and vs. MCF7. Data reduction employed *t*-testing ($p < 0.05$) and heatmap profiling using GeneSifter, applying a >twofold filter to determine kinase expression altered in each EGFR+ or EGFR− acquired TN phenotype, or deregulated in all TN models. Differentially-expressed gene lists underwent Ariadne Pathway Studio ontological analysis. Results: We cloned 2 novel acquired resistant human breast cancer models, EGFR+ 22.2 and EGFR− 1.2, which had gained an adverse TN phenotype during antioestrogen treatment since they lacked HER2, had lost ER and PR mRNA/protein, showed no antihormone, trastuzumab or EGFR inhibitor response, and had increased growth and invasiveness. 146 kinase genes were deregulated in the TN models. Along with EGFR, 7 were increased in EGFR+ 22.2 including receptor tyrosine kinase (RTK) FGFR4, with 27 increased in EGFR− 1.2 including RTKs FGFR2 and MET. 50 further kinases were increased in both TN lines, including RTKs STYK1 and TYRO3 and also enrichment of cell cycle/mitosis and DNA repair-associated kinases. RT–PCR has to date focussed on verifying the RTKs, confirming their induced expression profiles in the TN models. Conclusions: These models comprise a new resource to discover signalling potentially driving TN tumour phenotypes acquired by some patients during antihormonal treatment. Significantly, our gene expression studies have determined that FGFR2, FGFR4, MET, STYK1 and TYRO3 (RTKs with growth and invasion ontologies) are worthy of continued investigation to determine if they comprise novel signalling targets for inhibition of acquired TN breast cancers.

P025-A: Modelling Faslodex response in vitro to maximise drug effectiveness

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Introduction: The antihormone tamoxifen and aromatase inhibitors provide a mainstay in treating early and advanced ER+ breast cancer, while Faslodex (fulvestrant) is currently approved as a second line antihormone. However, initial response to antihormones are variable, complete response is rare, and resistance is eventually acquired by many patients. It is important to model these events to determine mechanisms underlying antihormone response and failure so targeted strategies can be developed to maximise drug effectiveness, but to date studies have primarily employed MCF7 cells and their derivatives which cannot represent the breadth of ER+ clinical disease. Focusing on Faslodex, our objective here was to use 4 cell lines in order to reflect major ER+ clinical phenotypes (ER+/HER2− MCF7, T47D; ER+/HER2+BT474, MDAMB361) to: (i) characterise in vitro magnitude of initial response and monitor onset of resistance by prolonged treatment and (ii) detail gene expression changes during Faslodex treatment.

Experimental procedures: Cell models were grown in the presence of Faslodex until resistant sub-populations emerged. In parallel, total RNA was isolated from untreated and 10 day Faslodex treated cells to carry out gene microarray analysis using the Affymetrix Human Genome U133 Array GeneChips. Transcriptome changes were determined using online and in-house bioinformatic tools including GeneSifter, and Ariadne® Pathway Studio.

Results: All models were initially growth-inhibited by Faslodex, with superior responses in HER2− lines. In parallel, gene microarray analysis revealed that while Faslodex both suppressed and induced genes in all models, its target cohorts generally differed between HER2+ and HER2− models. Interestingly, while MCF7, BT474 and MDAMB361 eventually acquired Faslodex resistance, this failed to develop in T47D providing a model for complete response. Similarly, the gene cohorts influenced by Faslodex in the complete responding HER2− T47D line showed further differences both to the HER2− MCF7 and HER2+ lines, a disparity augmented following treatment. In order to begin to determine Faslodex-deregulated genes that may permit subsequent acquisition of resistance, pathway enrichment was conducted on gene cohorts showing an equivalent expression profile with Faslodex in BT474, MDAMB361 and MCF7 using Ariadne software, also filtering for >twofold change with Faslodex, expression call and adverse ontology. 5 genes emerged as potential cell survival elements induced by Faslodex in all three models: GRN, PPP3CA, PRKACB, KITLG and VEGFC. Filtering based on >twofold change and ontological analysis was also used to determine Faslodex-deregulated, potential apoptotic or anti-proliferative genes unique to T47D cells that may facilitate the complete Faslodex response in this model. 4 genes were identified: DCN, PCDH7, CASP1 and ADAM12.

Conclusions: Using models spanning ER+ HER2− and ER+ HER2+ phenotypes, genes have been identified that may permit cell survival or underlie growth inhibition during Faslodex treatment of ER + breast cancer and could prove useful predictive biomarkers. PCR verification and gene knockdown/overexpression studies will now be performed for the genes of interest to consolidate whether their signalling comprises novel targets to maximise Faslodex response.

P026-B: Computational analysis of chromatin landscape in breast cancer genome based on ChIP-seq data of histone modifications

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To study gene expression regulation and interplay with chromatin modifications in breast cancer genome we developed set of computer tools for analysis of ChIP-seq data. Epigenetic modifications are important in regulation of gene expression. Chromatin landscape and, in particular, histone H3 modifications, have cell-specific effects on availability of TF binding sites and recruitment of RNA Pol II complexes. Recent studies have demonstrated chromatin remodeling even in terminally differentiated normal cells and advanced-stage melanoma and breast cancer cells, in context-dependent responses to

alterations in their microenvironment. Using advances in high throughput sequencing technologies in combination with chromatin immunoprecipitation (ChIP-seq), we constructed and analyzed chromatin modifications maps in MCF-7 and T47D breast cancer cell lines for activation marks, H3K4me1, H3K4me3 and H3K27me3 repressive marks. We further analyzed these in conjunction with genome-wide binding profiles of ER, FOXA1 and p53. Estrogen receptor (ER) is regarded as a major factor in breast cancer and FoxA1, transcription factor has been found to function as a pioneer factor in the recruitment of ER to gene regulatory regions. We used extensive search of co-motifs in regions proximal to ChIP-seq binding peaks in reference human genome. Binding motifs of transcription factors AP-1, SP1 and E2F1 were found among co-activation factors. To show that this was an unbiased analysis of genome-wide maps we developed software to test statistical significance of associations between chromatin modifications. The impact of chromatin configuration (as combinations of histone modification marks) in determining both ER binding site selection and their association with gene induction or repression by ER was explored by statistical tools. Predictive logistic regression model for ERalpha binding was constructed. Clustering of regions with activating and repressive histone marks in MCF-7 genome revealed strong co-localization for several histone modifications. Positive correlation between binding intensity of ERalpha and open chromatin conformation (activation histone marks) was shown by statistical measures. In more general approach we studied association between nucleosome occupancy (predicted by DNA sequence and experimentally obtained by sequencing) and affinity of TF binding measured by ChIP-seq. Network analysis of cancer-related genes within 10 kb of ERalpha binding sites was fulfilled using STRING protein interaction database, Ingenuity Pathway Analysis (IPA) and SkyPainter Interactome software. We found clusters of genes having common chromatin activation pattern. Together, our data shows that TF binding following gene activation in cancer genome is associated with chromatin modifications specific for cell lines that could be used as powerful predictive tool.

P027-A: Surface plasmon resonance-based biosensors for hormone receptor biology

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Estrogen receptors (ERs) are ligand-activated transcription factors that play key roles in many physiological processes and are one of the most important nuclear hormone receptors in breast cancer biology. When hormone receptor proteins capture estrogens, the receptors move to the nucleus and regulate gene expression by binding to specific DNA sequences known as estrogen response elements (ERE). Because small changes in nucleotide composition of these DNA strands can have large effects on transcription rate of target gene, the ability to detect changes in hormone activity with DNA-level accuracy is vitally important.

We have applied two surface plasmon resonance (SPR) techniques to study ER-ERE interactions, namely planar gold film-based SPR spectroscopy and gold nanoparticles (AuNPs)-based localized SPR technique. For the planar gold film-based SPR spectroscopy, we have designed a series of assay schemes to characterize a wide range of ER-ERE binding properties, including binding affinity, sequence specificity, stoichiometry, sequence-independent transient binding, distinct binding properties of protein subtypes, etc. To facilitate the high-throughput screening activity, we have developed a simple and fast AuNPs-based assay to study ER-ERE interactions. The assay is based on the unique optical properties of AuNPs arising from the localized SPR phenomenon. ER-ERE binding in homogenous solutions was measured based on interactions with the AuNPs and modulation of the particlesâ€™ optical properties (i.e., LSPR spectrum and solution color). The sensitivity of current assay enables single variations in the ERE core sequences to be colorfully and instantly detected.

We have benchmarked the AuNPs-based assay to the conventional planar Au film-based SPR technique. The parallel studies of the same biological systems using these two techniques allow us to compare the performance of the solid–liquid phase and homogenous phase assay modes and to comment on their suitability for fundamental research and for high throughput screening.

P028-B: PRMT4 plays a role in the proliferation of both hormonally dependent and independent breast and prostate cancers cells

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The onset of both breast and prostate cancers are multi-factorial events that often depend on steroid signaling pathways downstream of the estrogen/progesterone/Her2 receptor (breast) and androgen receptor (prostate). The progression of prostate cancer however, often involves a molecular switch where the cancer changes from an androgen dependent tumor to an androgen independent tumor leading to treatment resistance, while in breast cancer, the largest unmet medical need lies in the treatment of triple negative (ER/PR/Her2 negative) tumors that are unresponsive to current therapy. This necessitates the search for novel targets that may be effectively targeted in these hard to treat cancers.

PRMT4 (CARM1) belongs to a family of arginine N-methyltransferases enzymes that catalyze the transfer of a methyl group from S-adenosyl-L-methionine to the side chain nitrogens of arginine residues within proteins to form methylated arginine derivatives and S-adenosyl-L-homocysteine. Specifically PRMT4 methylates core histone proteins on H3 and H4, which are also targets of histone acetylase activity of CBP/p300 coactivators. Recently, PRMT4 epigenetic activity has been implicated in signal transduction, and transcriptional regulation in the onset and progression of steroid stimulated breast and prostate cancers. As treatment regimes for hormonally dependent breast (e.g. Tamoxifene) and prostate (surgical or chemical castration) cancers already exist, our interest was to explore if PRMT4 activity played a role in the proliferation of both hormone dependent and hormone independent breast and prostate cancers.

Our initial study showed that the transient siRNA knock-down of PRMT4 expression led to a strong decrease in cell proliferation in both un-stimulated ER positive (MCF7) and ER negative (MDA-MB231) breast cancer cells. Follow-up studies in un-stimulated breast cancer cells utilizing doxycycline inducible, stable lenti-viral vectors (pSlik) targeting PRMT4 corroborated the strong decrease in cell proliferation associated with transient (siRNA) PRMT4 knock-down. Furthermore, knock-down of PRMT4 led to a dramatic decrease in Histone H3-arginine 2-di-methylation (H3-R2-Me2) mark in the same

cells, suggesting that the loss of proliferation in breast cancer cells is associated with the loss of PRMT4 enzymatic activity. In addition, we showed that inducible knock-down of PRMT4 in 17-estradiol stimulated MCF7 (PRMT4-pSlik) cells led to a significant decrease in proliferation, confirming previous publications that PRMT4 plays a role in ER dependent breast cancer proliferation. In addition to breast cancer, we also showed that knock-down of PRMT4 was associated with a strong decrease in proliferation in androgen independent LNCAP prostate cancer cells utilizing the same doxycycline inducible pSlik system.

This initial data suggests that PRMT4 plays a strong role in the proliferation of hormone independent breast and prostate cancers. The development of future therapies targeting PRMT4 may be beneficial to patients with hormone independent breast and prostate cancers.

P029-A: Transcriptome and miRNA analyses of mouse and human mammary epithelial subpopulations

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Molecular characterization of the normal epithelial cell types is an important step towards understanding pathways that regulate self-renewal, lineage commitment and differentiation along the cellular hierarchy. In this study we carried out genome-wide mRNA and microRNA expression profiling in four distinct murine and human subpopulations: stem cell-enriched, luminal progenitor, mature luminal and stromal cells. All the mouse mammary epithelial subpopulations exhibited distinct gene expression and microRNA signatures, which are substantially conserved with their human counterparts. The Ingenuity Pathway Analysis (IPA) also identified a number of conserved pathways across species. This study further validates the use of the mouse as a model to study mammary gland development and highlights pathways that are likely to govern cell-fate decisions and differentiation.

P030-B: In vitro and in vivo effects of ceramide on dysfunction of apoptosis in breast cancer

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Background and purpose: Today, breast cancer is one of the most commonly diagnosed cancers in women. It is well known that dysfunction in the physiological pathways of programmed cell death may promote proliferation of malignant cells whereas correction of such defects could selectively induce apoptosis in cancer cells. Sphingolipids, which include ceramides and sphingosine, are essential structural components of the cell membranes. They have cell messenger function, which regulates cancer cell proliferation, survival and death. Previous studies have suggested that exogenous and cell-permeable ceramides may be used as an adjuvant to chemotherapeutic agents for the treatment of multiple cancers.

Thus, the aim of the present study is to investigate the in vitro effects of exogenous ceramide on the induction of apoptosis in MCF-7 cells (breast cancer) as well as to investigate its in vivo effect on a chemically induced breast cancer model in female Sprague–Dawley rats.

Method: Mammary carcinogenesis was initiated by a single, intravenous (i.v.) tail vein injection of 7,12 dimethylbenz(α)anthracene (DMBA) at a dose of 5 mg DMBA/2 ml corn oil/kg body weight in female Sprague–Dawley rats at 7 weeks of age. Ceramide supplementation was started 5 days in week, 2 weeks prior to DMBA injection and continued for 24 (31 weeks of animal age) weeks and 35 (42 weeks of animal age) weeks of post DMBA injection. Histopathological, immunohistochemical and morphological studies were performed such as cell proliferation assay, apoptosis assay, western blot analysis, TUNEL Assay, annexin V apoptosis Assay, caspase 3-like activity assay & AKT. Agarose gel electrophoresis was done to detect the DNA fragmentation. Mitochondrial function was detected by MTT assay. mRNA expression of Bcl-2 family gene members were determined by reverse transcription polymerase chain reaction (RT–PCR).

Results: Ceramide induced apoptosis was observed in MCF-7 cells at 0, 8, 16, and 24 h, and the cell viability was detected & cell counting was analyzed using ELISA. After exposure to 20 μmol/L of C6-ceramide for 8 and 24 h, cell apoptosis was found to be 54.1% and 85.3% respectively. This showed a time and dose-dependent relationship. The detection of apoptosis was done by fluorescein isothiocyanate labeled annexin V followed by flow cytometric analysis of the apoptotic cells. After C6 ceramide treatment, typical characteristics of apoptosis, such as nuclear chromatin breakage, apoptotic body and DNA ladder, could be observed. Meanwhile, ceramide up-regulated or down-regulated the mRNA expression of Bcl-2 family gene members with reduced antiapoptotic phosphorylation of the Akt. Administration of ceramide further showed a prominent reduction of cell proliferation (27.44%, $P < 0.001$) and an increased expression of p53 protein (3.34 ± 0.11, $P < 0.001$) in preneoplastic mammary tissue when compared to carcinogen control counterpart. Histopathological and morphological analyses were carried out as end-point biomarkers.

Conclusion: This study shows that C6-ceramide may have potential anti-carcinogenic effect in both in vitro and in vivo mammary carcinoma by affecting cellular proliferation and apoptosis.

P031-A: Hedgehog ligand overexpression predicts poor outcome in breast cancer and is a potential therapeutic target for metastatic disease

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The Hh signalling pathway plays an important role in a number of malignancies, and accumulating evidence suggest it contributes to the development and progression of breast cancer. However, there is

relatively scant data regarding the clinical significance of Hh pathway dysregulation in breast cancer or its contribution to malignancy in vivo. We investigated the expression of Hh pathway components in a well-characterised cohort of 279 patients with invasive ductal carcinoma (IDC) to determine its prognostic significance and found high Hh ligand expression was associated with increased risk of breast cancer recurrence (HR 1.95, $p = 0.0004$) and breast cancer specific death (HR2.3, $p = 0.0002$). High Hh was also strongly associated with the basal-like phenotype. Furthermore high Hh expression is an early event in the development of breast cancer, and was observed in two independent histological progression series ($n = 301$) to increase with greater atypia and malignancy of pre-invasive lesions. Hh ligand overexpression in a mouse mammary carcinoma model resulted in a fourfold increase in tumor size, and was associated with increased proliferation, shorter time to metastases and shorter survival times ($p = 0.0001$).

Furthermore, tumors overexpressing Hh were histologically more poorly differentiated than control tumors. Hh overexpression in vivo was associated with robust upregulation of canonical Hh target genes Ptc2, Gli1 and HHip. Cellular fractionation of tumours revealed that gene expression was activated in stromal cells, rather than the neoplastic cells, indicated a paracrine mechanism for Hh action.

To determine whether Hh is a potential therapeutic target for breast cancer, we treated tumor-bearing mice with the Hh neutralizing monoclonal antibody 5E1. Inhibition of Hh signaling caused a dramatic reduction in tumour growth rate and metastatic dissemination compared to mice treated with control antibody. Using an aggressive metastatic mammary carcinoma cell line model (4T1) that endogenously expresses Hh ligand, treatment with antibody 5E1 also resulted in reduced lung metastatic burden ($p = 0.045$).

In conclusion, we report that high Hh ligand is associated with a poor prognosis in IDC and is an early event in breast cancer development. Overexpression of Hh ligand promotes mouse mammary tumor growth as well as shorter time to metastatic disease and death, via paracrine signalling to adjacent stroma. Finally, we demonstrate that blockade of the Hh ligand is a potential therapy in metastatic breast carcinoma. These data are all unpublished.

P032-B: Deforming DNA to regulate the genome: A FRET study on the Sox family

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Despite binding to near-identical DNA sequences with high affinity, individual Sox proteins regulate the expression of distinct sets of genes leading to diverse cell fate decisions. Importantly, Sox proteins bend DNA by interacting with the minor groove of the double-helix. DNA bending may be a crucial act to assemble specific regulatory complexes and different bending angles may result in variant transcriptional outcomes. Here we asked whether individual Sox family members deform DNA in a distinctive manner to facilitate the subsequent formation of Sox-specific regulatory complexes. To this end we measured the FRET efficiency of fluorescently labeled DNA before and after protein addition. After determining the distances of the DNA ends we were able to compare bending angles induced by Sox2, Sox4, Sox5, Sox7, Sox17 and Nanog at saturating concentrations. We found that all Sox proteins significantly shorten end-to-end distances consistent with a bending angle of $\sim 70^{\circ}$, but the homeodomain protein Nanog showed no bending effects. Notably, while the other Sox proteins deformed DNA in an indistinguishable manner, Sox 5 was found to bend DNA significantly less strongly. Furthermore the influence of cation concentrations and variations in the DNA sequence on the bending angle was investigated. We conclude that even though differential bending does not suffice to explain the multitude of biological processes regulated by Sox proteins subtle differences as seen for Sox5 may contribute to its specific biological role.

P033-A: Identification and functional analysis of AP2γ as a novel transcriptional cofactor of estrogen receptor α in breast cancer

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Estrogen receptor (ER) is important in the development and progression of breast cancer. Using ChIP-sequencing, we mapped the genomic landscape of ERα binding sites (ERBS) in the breast cancer cell line, MCF-7. To understand what factors can influence the transcriptional activity of ERα, we performed cofactor motif analysis on the ERα binding sites identified by ChIP-sequencing. Our analysis revealed the binding sequences of AP2 family of transcription factors were highly enriched in the ERBS. The AP2 family of transcription factors is known to orchestrate a variety of cellular processes, including cell growth, cell adhesion and tissue differentiation. AP2γ, a member of the AP2 family, has been shown to be over-expressed in breast tumors, however little is known on how it regulates transcription in breast cancer. Here, we showed that AP2γ is recruited to the distal ERBS of the estrogen regulated gene, *re*arranged during *t*ransfection (RET), in a ligand-independent manner. Using a combination of siRNA, ChIP and Chromosome conformation capture (3C) assays, we showed that AP2γ is essential for the recruitment of ERα and FoxA1, and the long-range chromatin interaction at the RET gene. Moreover, down-regulation of AP2γ resulted in reduced RET transcription. Taken together, our data suggests that multiple transcription factors (e.g. AP2γ and FoxA1) are pre-loaded at ERBS establishing functional complexes of cofactors that are required for the coordinated regulation of ERα activity.