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Oral Presentations

O1: Application of whole exome sequencing for the identification of breast cancer susceptibility genes

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The ability to identify disease-causing mutations in high risk breast cancer families has broad implications for those affected, in terms of risk assessment and management as well as treatment. With as much as 75% of the excess familial risk of breast cancer yet to be attributed to genetic variants it is likely that additional moderate to high risk breast cancer genes remain to be discovered. Recent advances in technology have opened up the possibility of using massively parallel short-read (or 'next generation') sequencing to uncover predisposing mutations in individuals with inherited cancer in an unbiased manner.

We are performing whole exome sequence analysis of germline DNA from multiple affected relatives from over 75 high risk non-BRCA1/non-BRCA2 breast cancer families with the aim of identifying segregating, rare, non-synonymous variants that are likely to include novel predisposing mutations. Initial experiments using the NimbleGen Human Exome Array (to capture 180,000 coding exons and 551 miRNAs) and the Illumina GAIIx platform (combining 75 bp single end data from three flow cell lanes) generated approximately 3 Gb of mappable sequence data with 54% target specificity. The average fold-coverage within the target regions was 60X and importantly, 95% of targeted nucleotides were covered by at least eight sequence reads. Single nucleotide polymorphisms (SNPs) are identified using MAQ-SNP and short insertions and deletions (indels) are identified using novoalign. After excluding all variants reported in dbSNP, an average of 5 novel nonsense SNPs and 20 frame-shift indels are identified per index case. The number of nonsense mutations and frameshift indels shared by affected relatives further reduces the candidate gene list to less than 5 per family.

To date, two potential breast cancer predisposing genes have been identified. Interestingly, in two families, we identified clearly deleterious mutations in BRCA2 and PTEN, respectively. In the BRCA2 family, the index case used for diagnostic BRCA2 sequencing was a phenocopy and did not carry the mutation. In the case of PTEN, this gene is known to predispose to breast cancer but curiously none of these family members showed clinical manifestations of Cowden's syndrome. Since PTEN mutation testing is only performed in breast cancer families if there are Cowden's syndrome features, it is possible that PTEN mutation may be under-reported.

Our experience suggests that a proportion of unexplained BRCAx families may be due to already recognised predisposing genes that have been missed either because only a single index case and/or only one or two of the predominant genes have been sequenced. Both these limitations are enforced due to the current prohibitive cost of conventional mutation detection strategies used in diagnostic laboratories. Using targeted exome capture it will be possible to simultaneously sequence all of the top breast cancer predisposing genes in multiple individuals of a family more rapidly, and at a cost that is less than those currently incurred when sequencing a single gene like BRCA1. In summary, whole exome sequencing of multiple individuals from within each cancer family is proving to be an efficient strategy for rapidly identifying novel familial predisposing mutations.

O2: Using preimplantation genetic diagnosis to avoid the inheritance of predisposition to breast cancer

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Mutations in the genes BRCA1 and BRCA2 are known to be responsible for increased risk for breast and ovarian cancers and contribute significantly to an inherited predisposition to breast and ovarian cancers. Studies suggest an estimated cumulative risk of breast and ovarian cancer in female gene carriers of 87 and 44%, respectively (BRCA1) and 84 and 27%, respectively (BRCA2) by age 70, while male carriers reportedly have an elevated risk of prostate or breast cancers. Preimplantation genetic diagnosis (PGD) can be used to identify and select for embryos not carrying these mutations.

Nine couples were referred for PGD where the female or her partner was a known carrier of either a BRCA1 or BRCA2 germ line mutation. All couples had consultations with an infertility specialist, geneticist, nurse, scientist and counsellor to ensure informed consent to the procedures. The PGD tests comprised a number of informative

polymorphic linkage markers in or around the gene and, where possible, a specific mutation. Five BRCA1 and 3 BRCA2 couples (average ages of 29.1 years and 35.0 respectively) proceeded to clinical treatment.

The five BRCA1 couples had a total of 7 IVF-PGD cycles and 2 freeze–thaw cycles. Forty embryos were tested with 28 embryos identified as mutation free; 6 unaffected embryos were transferred in 5 fresh transfers (2 cycles were freeze-all cycles). A total of 22 embryos were frozen. In addition, 2 embryos were transferred in 2 freeze–thaw cycles. Overall 4 couples achieved an ongoing pregnancy (including one twin pregnancy) giving a pregnancy rate of (4/7) 57% per PGD cycle started, and an implantation rate of (5/8) 63%. The three BRCA2 couples had a total of 3 fresh IVF-PGD cycles. Two couples only had 1 and 2 oocytes, respectively and so did not proceed to PGD biopsy and analysis. The remaining couple had 13 embryos tested with 8 embryos identified as mutation free. One unaffected embryo was transferred and 7 embryos were frozen. The transfer resulted in a healthy live born.

PGD provides a viable clinical option to select embryos without the known, serious cancer predisposition mutation. It can be an effective and efficient treatment enabling couples to achieve a pregnancy free of an inherited BRCA mutation and thus reduce the psychological burden of consideration of pregnancy termination as well as eliminating the potential need for future radical prophylactic surgery for a female child.

O3: Differentially expressed genes in ductal carcinoma in situ and invasive ductal carcinoma compared with normal breast tissues

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In order to identify differentially expressed genes in breast cancer tissues, we performed microarray hybridizations and analysis of 80 breast tumor RNAs against pooled normal RNA (20 RNAs) using the Agilent 44 K Oligonucleotide arrays. Amongst these, six tumors were DCIS. A total of 6,689 genes are differentially regulated with a fold difference of 1.5 ($P = 0.01$) in DCIS compared to normal breast tissue. Amongst these 3,282 are up regulated and 3,407 are down regulated. Comparison of DCIS expressed genes with IDC node positive tumors revealed a total of 7,004 differentially expressed genes as against 8,001 genes in node negative tumors. Interestingly DCIS expressed genes forms a distinct cluster in comparison to other tumors. Several genes previously not known to be involved in breast cancer showed differential expression in DCIS. There are several commonly expressed genes between DCIS and IDC including some of the genes known to be involved in invasion suggesting the expression of invasive gene signature early on in breast tumorigenesis. The data along with validations will be presented. To the best of our knowledge this is the first data set in Indian breast cancer patients describing gene signatures.

O4: Her2 and the synergistic binding of its antibodies: in silico insights

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The ErbB family of receptor tyrosine kinase consists of 4 members, EGFR, Her2, Her3 and Her4. Her2 is amplified/overexpressed in about 20% of breast cancers and represents an important therapeutic target in this subset of patients. The use of monoclonal antibodies targeting Her2 is one of the most efficacious therapeutic approach in the clinical setting. These molecules include trastuzumab, binding to domain IV of the Her2 ectodomain and pertuzumab, binding to the dimerization arm (domain II).

The combination of these two Abs has recently shown unexplained synergism in clinical trials, where the efficiency over trastuzumab alone was greatly enhanced.

Computational structural models of the interactions of her2 with these two antibodies suggest a feasible mechanism to support the observed synergism and will be discussed. Mutation studies to test this hypothesis are ongoing.

O5: Investigation of intracellular cancerous biomarkers: in silico analysis

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Cancer is a complex disease wherein the comprehension and elucidation of networks involving numerous interconnected pathways remains a challenging and important task. Over the past 50 years, a lot of data have been amassed via in vitro, in vivo experiments. The phenotypic identification of different cancers has proceeded well - however, very little knowledge of this has been utilized until now to figure out specific cancerous biomarkers. One key problem has been that these experiments are not always informative enough to extract the interactive effect of multiple pathways. In this regard, mathematical modeling techniques can facilitate in the development and analysis of the network models from the existing historical data of different intracellular processes regulating cell division process.

In this work, we developed an integrated mathematical framework incorporating different intracellular pathways such as glucose metabolism, p53 activation, activation of kinase complexes during cell cycle. Then, we applied global sensitivity analysis techniques on the model to find the key parameters affecting the unregulated and uncontrolled cell division. These results can perhaps indicate opportunities in the field of drug design for the implementation of personalized therapies. In summary, these kind of in silico based studies can provide supporting tools for investigating influential mechanisms controlling the cancer growth and testing different hypotheses.

O6: Silicon nanowire biosensor for detection of protein-DNA interactions in nuclear extracts from breast cancer cell

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Silicon nanowires (SiNWs) biosensor, as a label-free and highly sensitive electronic detection methodology, has been extensively

developed for detection of nucleic acids and proteins. However, little is known about studying of protein-DNA interactions using the SiNW biosensor. In this work, a self-assembled monolayer (SAM) was formed on the SiNW surface, and the SAM-assisted SiNW biosensor was capable of characterizing the protein-DNA interactions with good specificity and sensitivity. More importantly, the SiNW biosensor was able to detect the protein-DNA interactions in nuclear extracts from breast cancer cell.

As a proof-of-concept study, estrogen receptor element (ERE) and estrogen receptor alpha (ERÎ±) binding was adopted in the work. The ERÎ± contains positive charges in neutral HEPES buffer because pI of ERÎ± is approximately 8. The contribution of positive charge to the n-type SiNW surface induces an increase in conductance. Three sequences of the EREs were employed in this study. The wild-type ERE (wt-ERE) carries the palindromic GGTCA half-site with a 3-bp separation. The mutant ERE (mut-ERE) contains a symmetric base substitution in each of the ERE half-sites. The scrambled-ERE (non-ERE) has the sequence in the ERE arms scrambled. Specificity of ERÎ±-ERE binding was obtained by applying 1 pM ERÎ± to the three ERE-functionalized SiNW surface. A remarkable conductance increase was obtained when the ERÎ± was bound to the wt-ERE-functionalized SiNW biosensor, whereas a negligible response was observed when the ERÎ± interacted with the non-ERE. To evaluate the capability of the SiNW sensor for discrimination between mut-ERE and wt-ERE, the mut-ERE-functionalized SiNW sensor was incubated with ERÎ±. The increase in conductance was much lower than that of wt-ERE. The high specificity suggests that the unique SAM-assisted SiNW biosensor allows for label-free discrimination between the wild type and mutant EREs interacting with ERÎ±. Subsequently, sensitivity was investigated by applying various concentrations of ERÎ± from 1 pM to 1 fM to the wt-ERE-functionalized SiNW sensor. It was observed that the conductance change dropped as a function of varying concentration of ERÎ± and the ultralow concentration of ERÎ± could be effectively detected down to 10fM with the SiNW biosensor. ERÎ±-DNA interactions in nuclear extracts prepared from MCF-7 breast cancer cells were also investigated. The ERÎ± knocked down by siRNA in the same cell line and no ERÎ± from another negative cell line (MDA MB 231) gave rise to negligible response.

In this presentation, a SAM-assisted SiNW biosensor has been developed for the specific and high sensitive characterization of protein-DNA interactions. The SiNW biosensor is capable of distinguishing the base substitutions in ERE half-sites by binding them to ERÎ±, and the wt-ERE-functionalized SiNW biosensor is proven to detect 10 fM of ERÎ±. The biosensor is capable of detecting the protein-DNA interactions in nuclear extracts from breast cancer cell, shedding light on ER-mediated gene expression for breast cancer.