Oral Presentations (Mon, 26 Sep, 14:45−16:50)
Basic Science/Translational Research

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Luminal A Breast Tumours Divided in Two Clusters by DNA Methylation

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Background: Gene expression profiles and DNA methylation profiles have been shown to be of importance for breast cancer development and survival of patients. Based on gene expression profiles five groups of tumours have been identified (Luminal A, Luminal B, ERBB2 enriched, basal-like and normal-like) and the groups show different survival. Luminal A is usually the largest group and has the best prognosis, while basal-like has the worst prognosis. Lately, analyses have identified three groups based on DNA methylation (called Cluster 1−3) that also show different survival. Cluster 3 has best prognosis, Cluster 1 has an intermediate prognosis, while Cluster 2 has worst prognosis. The concordance between gene expression groups and DNA methylation groups is strong, though, interestingly, the Luminal A tumours are split quite evenly between Cluster 1 and Cluster 3. Based on the split between Cluster 1 and 3, patients having Luminal A tumours show different survival. This study set out to further investigate the two groups of Luminal A tumours.
Material and Methods: DNA material from 80 breast tumours were analyzed by Illumina GoldenGate interrogating 1505 CpGs, and 102 breast tumours were analyzed by Illumina Infinium 27K methylation array interrogating more than 27 thousand CpGs. Whole genome expression profile was available for all samples. The samples were collected at hospitals in Oslo/Akershus and all patients have given informed consent and the projects are approved by the local ethical committee.
Results: Using SAM analysis on Luminal A tumours, 41 genes were found differentially methylated between Cluster 1 and Cluster 3 (FDR < 5%), and these included *BIRC4*, *CD40*, *CDKN1C*, *EGFR*, *ESR2*, *ICAM1*, *KIT*, *MAS1*, *SFRP1*, *TERT*, *WNT1 and WT1*. Further, the gene list was used to do hierarchical clustering on Luminal A breast tumours analyzed by Illumina 27K methylation array, and also these tumours was split in two groups. When applying Kaplan–Meier survival analysis on these two groups, a significant difference was observed. Further results will also include biological analysis of the genes involved in separation of the two clusters.
Conclusions: Earlier work has shown that Luminal A tumours are split between two DNA methylation clusters and that these show different survival. Here we show which genes drive the separation of the Luminal A tumours into two groups by DNA methylation, and we show that the difference in survival is apparent on multiple dataset and analyzed on different platforms. We will also show a biological interpretation of the genes involved.

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Anti-cancer Therapy Mediated by a Histone Deacytelase Inhibitor Engages the Immune System

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Histone deacetylase inhibitors (HDACi) are novel anti-cancer agents, that potently induce tumour cell-specific apoptosis. HDACi are thought to enhance tumour cell immunogenicity and promote engagement of immune effector mechanisms that can contribute to the anti-tumour activities of the compounds. We therefore hypothesised that combining HDACi with immunotherapy could provide enhanced therapeutic efficacy when tested in preclinical murine models of carcinoma and lymphoma. Indeed, while the HDACi vorinostat could inhibit the growth of established colon, breast and renal carcinomas in vivo, anti-tumour activity was significantly enhanced when vorinostat was co-administered with immunomodulatory monoclonal antibodies to 4−1BB and CD40 (56%, 25% and 25% complete regressions respectively). Further investigation determined vorinostat induced an immunogenic form of tumour cell-specific apoptosis and enhanced phagocytosis of tumour cells by antigen presenting cells in vitro, together suggesting an inherent capacity of vorinostat to enagage the immune system. We have also shown vorinostat alone significantly prolongs survival of mice bearing Eμ-Myc B cell lymphoma compared to controls (20 days). However, this survival advantage was not observed in immunocompromised Rag-$2^{-/-}$common-$\gamma^{-/-}$, RAG1 and CD8-depleted mice (7.5, 8 and 12.5 days). Equivalent survival was observed when immunocompetent and Rag-$2^{-/-}$common-$\gamma^{-/-}$ mice were treated with etoposide (29 and 32 days), demonstrating the specificity of vorinostat-mediated immune engagement during anti-cancer treatment. Finally, treatment of immunocompetent mice with vorinostat bearing Eμ-Myc B cell lymphoma resulted in altered numbers of immune cell subsets at sights of primary tumour burden (spleen and lymph nodes), suggesting a further mechanism by which HDACi mediate anti-cancer activity. These novel data demonstrate an important requirement for adaptive and possibly innate arms of the immune system in HDACi-mediated anti-cancer therapy. Future studies will characterise the mechanism of HDACi-mediated effector cell engagement and activation in mouse models of cancer, and this information aid in the design of more potent combination strategies utilizing HDACi's.

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Senescence Induction by BRAFV600E is Associated With the Induction of Autophagy and Targeted Degradation of BRAF and CRAF

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More than 90% of *BRAF* mutations in human cancer are represented by a valine to glutamate mutation at residue 600 known as the ^{V600E}*BRAF* mutation. Our laboratory has generated mouse models expressing a conditional knock-in allele of $BRaf^{V600E}$ and in previous work we have shown that expression of the oncogene in a range of tissues including embryonic fibroblasts (MEFs), lung tissue, melanocytes and gastrointestinal crypts induces ~10 population doublings after which senescence ensues. At senescent time points we have found that the expression level of both BRAF and CRAF proteins is significantly reduced and this occurs concomitantly with a drop in ERK1/2 phosphorylation. We have further investigated the mechanisms underpinning this drop and, through qRT-PCR experiments, have found that it cannot be accounted for by down-regulation of the corresponding mRNAs. Furthermore, inhibition of the proteasome does not lead to a rescue in the expression level of the proteins. Our studies do, however, show that BRAF and CRAF accumulate in the insoluble fraction and, using LC-MS/MS, we have found that this accumulation is associated with phosphorylation of a novel serine residue at position 675 of BRAF. This unusual processing of the RAF proteins is associated with the induction of hallmarks of autophagy in the senescent cells as assessed by electron microscopy, GFP-LC3 aggregation and LC3II turnover. Thus, we propose that selective degradation of BRAF and CRAF proteins by autophagy can occur as a protective cellular response in the early stages of cancer development induced by ^{V600E}BRAF.

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Crosstalk Between Homeobox Proteins and Polycomb Complexes in P16INK4a Regulation

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Background: The *Ink4a*/*Arf* locus is the chromosomic region most frequently altered in human cancer. It encodes for two gene products ($p16^{INK4a}$ and ARF) that regulate the Rb and p53 pathways and which are key senescence effectors. Controlling the expression of the locus is critical to cell homeostasis. In normal circumstances, the locus is repressed by Polycomb repressive complexes (PRC), and its expression induced in response to stresses such as oncogenic signalling. How PRCs are recruited to their target genes, and specifically to the *Ink4a*/*Arf* locus is not fully understood, as there is no PRC core component with a DNA binding domain.
Material and Methods: We cloned 12 candidate transcription factors in retroviral vectors and expressed them in IMR90 human fibroblast to screen for their ability to regulate senescence by performing growth curves, BrdU incorporation and SA-β-Gal assays.
Results: Amongst the factors tested, we observed that expression of the homeobox-containing protein HLX1 extended cellular lifespan and delayed replicative senescence on human fibroblasts. Expression of HLX1 also blunted the growth arrest induced by Ras expression in an in vitro model of Oncogene-induced senescence. Expression of HLX1 did not significantly affect p53 or p21 levels, but when HLX1 was expressed we consistently