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GENE EXPRESSION PROFILING OF SUPERFICIAL BLADDER CANCER. Jeetesh M Bhardwa*, Mahesh Kumar, D M Berney, Joanne Martin, Finbar Cotter, Vinod Nargund.

Bladder cancer (TCC) is the sixth most frequent malignancy occurring worldwide. Superficial TCC accounts for 75-85% of all TCC. 70% of patients with superficial TCC have one or more recurrences after initial treatment, and about one third of these patients have progression of disease. Markers are needed to identify those most at risk of developing invasive disease.

A retrospective study of 128 patients with superficial TCC matched for age/ sex/ smoking habits and number of tumours on presentation were divided into 2 categories, those that had tumours that progressed into invasive disease and those which did not recur nor progress. mRNA extracted from archival material on these patients (n=4) was used to perform Gene expression analysis using the Affymetrix® Human Genome HG U-95A Gene Chip. Out of a total of 12,500 genes on the chip, 10% were called present. We have filtered these genes down to a total of 6 candidate genes, MMP11, ETV6, TFAP2, TNFRSF6B, TGFBIF2, ING1L that were over expressed in TCC that became muscle invasive, other solid tumours are also known to over express these genes There were also 7 genes ACVR1B, CDC2L2, SFN, RBBP8, BECN1, RPS29 and ABL1 that were under expressed in specimens that turned invasive, implying that these genes may have a role in tumour suppression.

We will validate our results by performing Real Time Quantitative PCR on the above-mentioned genes (n=20). We hope to be able to prove that the overexpressed genes play an oncogenetic role in invasive TCC while the under expressed genes are involved in tumour suppression and prevention of muscle invasion.

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A COMPARATIVE APPROACH TO CANCER CYTOGENETICS
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The clinical presentation, histology and biology of many canine cancers closely parallels those of human malignancies, and their extensive genome homology is well established. Comparative studies of related human and canine malignancies can make a significant contribution towards the understanding of tumour development in both species, and to improving tools for diagnosis, prognosis and therapy.Our ongoing studies focus on the molecular cytogenetic evaluation of canine cancers, the characterisation of non-random genomic abnormalities, and comparison with knowledge gained from more widely studied human counterparts. Ultimately this will lead to a greater ability to extrapolate data and resources between species, with potential benefit to both human and veterinary medicine.

As an example of this approach, data will be presented on preliminary comparative genomic hybridisation analysis of 25 cases of canine malignant multicentric lymphoma. This represents the most frequent life threatening cancer in dogs, comprising approximately 20% of all canine malignancies. Aberrations involved 32 of the 38 canine autosomes, with a maximum of 12 per case and a mean of three. Genomic gains were almost twice as common as losses. A subset of frequently encountered aberrations was detected. Potential correlations with immunophenotype and histological subtype have been observed. We aim to develop this approach for cytogenetic subdivision of this heterogeneous canine disease, and to correlate findings with clinical outcome. Comparisons may also be drawn with homologous aberrations observed in human lymphoma, suggesting that a related genetic aetiology maybe involved. This will form the framework for more detailed comparative studies.

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MOLECULAR CYTOGENETIC ANALYSIS OF A COHORT OF MEDULLOBLASTOMAS
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Although improvements in diagnosis and treatment of medulloblastoma (MB) have increased five-year survival to 50-60%, it is recognised that a clearer understanding of genetic alterations in MB may allow improved treatment stratification and suggest novel therapies. Using fluorescence *in situ* hybridisation, 44 MBs, histopathalogically classified as classic (CL;70.5%), anaplastic/large cell (A/LC;25%) or desmoplastic (DES;4.5%), were analysed for several genetic abnormalities known to occur in MB. 35% (15/43) showed a significant degree of polyploidy at more than 4 of 8 loci (47% being A/LC). Investigation of trisomy 7 indicated copy numbers between 3 and 7+ in 41% (18/44), while 8q24 (*MYC*), showed gain/amplification in 22% of cases. Analysis of 9q22.3 (*PTCH*), revealed 1 gain (DES) and 2 losses (CL and DES), while 10q23.3 (*PTEN*) and 10q24.31 (*SuFu*) showed paralleled losses in 3 cases (7%). Isochromosome 17q (i17q), the commonest aberration in MBs, occurred in 43% of cases and in 55% of A/LC. This is in agreement with previous reports. However, i17q was not preferentially clustered in A/LC MBs, as previously suggested, because 39% of CL MBs also showed this feature. In addition, 5 MBs had 17q gain alone. Although no clear associations between histopathology and molecular cytogenetics are immediately apparent, the results at present do confirm and question previous data. In particular, there is no close association between chromosome 17 abnormalities and the anaplastic phenotype.

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FUNCTIONAL ANALYSES OF ALTERNATIVE ISOFORMS OF PAX3 IN MELANOCYTES
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The paired box gene, PAX3 encodes a transcription factor, importantin cell proliferation, migration and survival during early embryonic neural and muscle development. Until recently, five different isoforms had been identified, while their functions remain unknown. We have discovered two new isoforms, g and h, lacking complete coding for exon 8. We have confirmed their sequences and subcloned seven isoforms, PAX3a-h into pcDNA4 expression vector. The constructed plasmids and empty vector pcDNA4 were stably transfected into a non-tumorigenic murine melanocyte cell line, Melan-a, using Transfectam. Transfected colonies wereanalysed for PAX3 isoform expression using specific primers bysemi-quantitative RT-PCR.

Promega Cell Proliferation Assay: melan a-PAX3h cells proliferated more rapidly than mock transfectants over 72h ($P<0.01$), while Melan a-PAX3a, -PAX3b cells proliferated more slowly ($P<0.01$)and Melan a-PAX3c, PAX3d, PAX3e, PAX3g showed similar rates of proliferation. A cell growth curve analysis over 7 days confirmed this, except that PAX3e grew more slowly than mock transfectants. Cell transformation assay: mock transfectants and cells containing PAX3a, PAX3b or PAX3e failed to form colonies in soft agar, whereas cells expressing PAX3c, PAX3d, PAX3g, and PAX3h formed colonies. PAX3d expressing cells formed larger colonies than the others, while cells having PAX3h produced more colonies. Together, our results suggest that alternative isoforms of PAX3 have different effects on melanocyte growth and transformation. Further studies are needed to determine whether there is a causal relationship between PAX3 isoforms and tumorigenesis in melanoma or whether one spliced isoform can regulate another, and to determine the molecular mechanisms involved.