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Scientific Abstract

SERRATED POLYPS, BRAF MUTATION AND COLORECTAL CANCER

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Background. Colorectal cancer (CRC) affects 1 in 23 people and is a leading cause of cancer mortality in Australia. The risks for developing CRC include increasing age, diet and genetic predisposition. Approximately 5-6% of CRCs are due to familial cancer syndromes, including hereditary non-polyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP). The majority of sporadic CRCs develop from adenomas and are characterized by aneuploidy and are microsatellite stable (MSS). The remaining 15% of sporadic CRC are diploid, rarely have *APC* or *p53* mutations and are associated with high levels of microsatellite instability (MSI-H) and thought to evolve via an alternate "serrated neoplasia" pathway. The MSI is due to epigenetic inactivation of a mismatch repair (MMR) gene, mainly *hMLH1*, caused by promoter hypermethylation associated with the CpG island methylator Phenotype (CIMP). Until recently the initiating events and precursor lesions responsible for MSI-H cancers were unclear. However, in 2002 a genome-wide mutational screen of cancers revealed the occurrence of oncogenic *BRAF* (V600E) mutation in ~10% of CRC (Davies et al. *Nature* 417 2002), and later shown to increase to 31% in MMR deficient CRC (Rajagopalan et al. *Nature* 418 2002) implicating *BRAF* mutation in the development of CRC. In addition, serrated (hyperplastic) polyps as opposed to adenomas were previously considered to have little or no malignant potential, but increasing evidence is now changing this view.

Results. We have performed two significant studies of *BRAF* and K-ras mutations in MSI-H cancers and serrated polyps. In the first retrospective study we investigated the frequency of *BRAF* anf K-*ras* mutations in a selected series of 145 cancers and 85 polyps (Kambara et al. *GUT* 53 2004). *BRAF* mutation was frequent in sporadic MSI-H cancers (76%) compared with HNPCC (0%). *BRAF* mutation was also frequent (77%), when cancers were stratified by CIMP. In polyps a high frequency of *BRAF* mutation was found in sessile serrated adenomas (SSA; 75%), and mixed polyps MP; 89%) compared with adenomas (0%).
In the second prospective study of 190 patients undergoing state-of-the-art magnifying dye-spray colonoscopy at the RBWH we investigated polyp incidence, location and type as well as *BRAF* and K-*ras* mutation (Spring et al *Gastroenterology* 131 2006). A total of 414 polyps

were detected in 72% of patients, comprising 60% adenomas, 29% hyperplastic polyps (HP) and 9% SSA, which tended to be large (64% >5mm). In the proximal colon adenomas (73%) and SSA (75%) were more prevalent. The presence of at least one SSA was associated with increased polyp burden and female gender. *BRAF* mutation was rare in adenomas (0.4%) but most common in SSAs (78%). K-*ras* mutation was associated with goblet cell HPs and tubulovillous adenomas.

Significance. The key finding of our research includes: (i) high frequency oncogenic *BRAF* mutation in sporadic MSI-H cancers and serrated polyps establishing a molecular link for the "serrated neoplasia" pathway in the development of MSI-H cancer and (ii) significant clinical implications involving (a) use of *BRAF* mutation as a diagnostic aid to exclude HNPCC when classifying MSI-H cancer and (b) emphasizing the importance of endoscopic identification and removal of large right-sided serrated polyps at colonoscopy for the prevention of colorectal cancer.

Lay Abstract

SERRATED POLYPS, *BRAF* MUTATION AND COLORECTAL CANCER

Colorectal cancer (CRC) affects 1 in 23 people and is a leading cause of cancer deaths in Australia. The risks for developing CRC include increasing age, diet and genetic factors. A small proportion (5-6%) results from syndromes caused by inherited mutations in specific genes, while the majority develop sporadically from polyps called adenomas. However, not all colorectal cancers develop from adenomas, but may also arise from another polyp type called hyperplastic or serrated polyps, Approximately 15% of sporadic cancers develop from serrated polyps via an alternate pathway known as the serrated neoplasia pathway.

Recently a gene called BRAF was found to contain a mutation (V600E) that appeared to associate with some cancers, including colorectal cancer. In our studies we looked for this mutation in different colorectal polyps and cancers and found a high frequency of BRAF mutation in particular types of polyps and cancers.

Our finding are important and it provides a genetic basis to the serrated neoplasia pathway and is changing clinical practice, including how to distinguish one type of colorectal cancer from another and identifying the importance of removing large right-sided serrated polyps during colonoscopy to prevent colorectal cancer.

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Scientific Abstract

OSTEAL MACROPHAGES: NOVEL REGULATORS OF BONE FORMATION

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Bone diseases are a major health problem and current treatments are inadequate. In particular there is a paucity of bone anabolic therapies that can rebuild bone in diseases such as osteoporosis. To aid development of such therapies, we need better understanding of the mechanisms regulating bone formation by osteoblasts (OB). Tissue macrophages are an integral component of many tissues and are important in homeostasis and repair. Macrophages have been demonstrated at sites of both pathologic bone deposition and loss, and can produce many known osteo-active factors. These observations link macrophages to bone homeostasis and disease and yet their role in bone biology is poorly understood. Using innovative *in situ* and *ex vivo* techniques, we have demonstrated that there is a population of resident tissue macrophages (referred to as OsteoMacs) present within both human and mouse bone lining tissues. Strikingly, OsteoMacs were also observed to be closely associated with areas of osteoblast-mediated bone formation. Notably, OsteoMacs formed an almost continuous cell layer over mature bone forming OB on cortical bone surfaces undergoing growth. Histomorphometric analysis demonstrated that 75.9±5.3% of the active OB bone surface was covered by this $F4/80^{+}$ OsteoMac 'canopy'. These observations demonstrate that OsteoMacs and OBs are intimately associated *in vivo* and suggest that OsteoMacs may regulate OB function. Primary OB cell preparations were demonstrated to contain a population of proliferating OsteoMacs. Removal of OsteoMacs from the OB preparations significantly decreased expression of the functional OB gene osteocalcin and their *in vitro* mineralization capacity in response to standard differentiation agents. We next examined the effect of OsteoMacs on OB functional responses to elevated extracellular calcium (eCa^{2+}), as eCa^{2+} is unique to the bone microenvironment and induces macrophage expression of the osteo-inductive molecule bone morphogenetic protein (BMP)-2. Using a co-culture system of macrophages with OBs, in either direct contact or separated using transwells, we demonstrated that OB mineralization occurred in response to eCa^{2+} only in the presence of macrophages. Conditioned medium from eCa^{2+} stimulated macrophages also induced mineralization in OB cultures, providing further evidence that macrophages produce a soluble factor that enhances OB mineralization. However, addition of BMP-2 blocking agents to these co-cultures did not inhibit macrophage-induced OB mineralization, indicating that BMP-2 is not required. These observations suggest macrophages detect changes in eCa^{2+} and consequently produce soluble factor(s) that drive OB mineralization. We have demonstrated

that OsteoMacs are an integral component of bone tissues and play a novel role in bone homeostasis through regulating OB function. This research challenges the dogma that calcium and bone homeostasis are primarily controlled by the balanced activities of osteoclasts and OB and is likely to reveal novel anabolic mechanisms that can be therapeutically replicated to improved treatment of bone disease.

Lay Abstract

OSTEAL MACROPHAGES: NOVEL REGULATORS OF BONE FORMATION

Over 3 million Australians suffer from bone diseases such as osteoporosis and osteoarthritis and their impact is expected to dramatically increase as a result of population aging. These conditions are now ranked third in the leading causes of health expenditure in Australia, with an estimated total cost of more than $3 billion per annum. Prevention and treatment of many bone diseases are currently inadequate. Consequently, there is a high demand for effective treatment options with a particular need for bone building (anabolic) agents. New anabolic treatments will only come from greater understanding of bone biology and particularly increased knowledge of bone formation. Our research provides greater understanding of the contribution of macrophages (cells important in tissue maintenance and immune responses) in bone growth, repair and disease. Macrophages have been implicated in many bone diseases, including osteoporosis and several forms of arthritis, linking aberrant macrophage function to bone damage. However the precise role of these cells in bone growth, repair and damage is poorly understood. We have shown that there is a significant population of macrophages intimately associated with bone, suggesting that these cells have a previously unrecognized role in normal bone biology. Strikingly, these cells have a particular affinity for sites of bone growth and formation. Using laboratory assays, we have also demonstrated that these macrophages enhance the function of specialized bone forming cells and that they are actually necessary for bone formation in response to certain bone growth signals. Our research supports that macrophages play a novel and important role in directing bone building. Further information on how these macrophages regulate bone formation will identify new ways of developing anabolic therapies. Bone building agents will drastically improve treatment options for people with bone diseases.

Dr Suyinn Chong

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Scientific Abstract

A PATERNAL EFFECT ON SEX CHROMOSOME STABILITY IN THE MOUSE

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Turner syndrome was first described in humans in the 1930s and is a chromosomal disorder that involves the absence of all or part of one sex chromosome. As a result, these individuals have a 45,X karyotype, are phenotypically female and display short stature and infertility. It has been estimated that this syndrome affects as many as 3% of all females conceived, however a large proportion (99%) spontaneously abort during the first or second trimester of pregnancy such that the frequency in liveborn females is approximately one in 2,000. In 60-80% of cases the retained X chromosome is of maternal origin, but it remains unclear whether the paternally derived sex chromosome is lost by a segregation error during gametogenesis (meiotic division) or early embryogenesis (mitotic division). Studies of the molecular mechanisms of sex chromosome aneuploidy have been hampered by difficulties in obtaining the relevant tissue in humans and the extremely low frequency of naturally occurring X monosomy in mice.

We are interested in the role of epigenetics in this process. Epigenetics is the study of heritable changes in gene expression that occur without a change in DNA sequence. DNA methylation is one type of epigenetic mark that is associated with transcriptional silencing. These marks are usually stable for the lifetime of an organism but are generally erased and reset between generations. We have shown that at some alleles the epigenetic marks can be transmitted across generations. Others have also reported that reduced genomic methylation *in vivo* can be associated with chromosome aneuploidy. In this study, we have used mice carrying a mutation in *DNA methyltransferase 3L* (*Dnmt3L*) to alter the epigenetic state of the gametes of the male parent and examined the consequences on sex chromosome ploidy in his offspring.

Dnmt3L is involved in the establishment of DNA methylation in germ cells and, in males, expression of *Dnmt3L* is restricted to diploid prospermatogonia, which undergo a number of mitoses before meiosis. Complete loss of Dnmt3L causes demethylation and increased expression of retrotransposons, followed by meiotic failure. Males haploinsufficient for Dnmt3L are phenotypically normal and fertile. We find that the offspring of males haploinsufficient for Dnmt3L have an increased frequency of sex chromosome aneuploidy.

Affected offspring have a 39,X karyotype (equivalent to Turner syndrome in humans) are phenotypically female and infertile. Interestingly, this chromosome instability is found in offspring that do not inherit the *Dnmt3L* mutation, and is therefore classified as a paternal effect. We propose that there is an epigenetic mark that has been altered in the male parent that in turn affects the sex chromosome constitution of their offspring. The sex chromosome that is lost can be of paternal or maternal origin. To our knowledge, paternal effects of this type have not previously been reported in mammals. Our findings show that the untransmitted genotype of male parents can influence the phenotype of their offspring, and represents a major shift in the way we think about the inheritance of traits in mammals. Our work may also provide insight into the aetiology of chromosomal aneuploidies and reduced fertility in humans.

Lay abstract

YOU INHERIT MORE THAN JUST DNA FROM YOUR FATHER

It is commonly believed that your physical appearance is determined by a combination of your genes, which are inherited from your mother and father, and the environment. My work, together with that of others, suggests that there is additional information attached to your genes, called epigenetic modification which is inherited from your parents. We have now found that the epigenetic modifications on the genes you inherit from your father can affect the stability of your sex chromosomes. We have shown in mice that reduced DNA methylation in the father's sperm increases the risk of chromosome loss in his offspring. Females normally have two X chromosomes, but we find some female offspring that have only one. These females are also infertile. Our findings in mice are very similar to a chromosomal disorder in humans called Turner syndrome. Turner syndrome females have a single X chromosome, short stature and infertility. It affects approximately one in 2,000 female births and the cause of this syndrome remains unknown. Our work provides new insight into the molecular mechanisms that determine your physical appearance and the possible origins of reduced fertility and chromosome loss in humans.

Dr Christine Wells

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Scientific Abstract

Genomic control of macrophage activation and inflammatory resolution

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Macrophages mediate much of the pathology of inflammation, particularly in the acute phase of an infection. The processes underlying inflammatory activation are relatively well documented and are the targets of most therapeutic interventions. Equally important but are often overlooked are the 'inflammation suppressors' - naturally occurring repressor molecules that are produced by acutely-activated macrophages to self-regulate inflammation. A new class of repressor molecules are isoforms of a canonical activating molecule that, through genomic regulatory mechanisms such as alternate splicing, have modified functions. There are *ad-hoc* examples of alternate splicing events regulating innate immune function, but our approach provides the first systematic analysis of these events in activated macrophages.

We have designed 'splicing arrays' to detect the expression of variant transcripts in macrophages responding to endotoxin. We demonstrate that the repressor versions of many signalling molecules involved in the Toll-like receptor pathway are detected in the acute response phase of an immune stimulus. Our data suggests that alternate splicing in macrophages occurs in response to signal transduction pathways, & requires inducible recruitment of splice factors as well as tissue-specific splicing regulators.

Our laboratory uses a 'systems biology' approach to map out the network of gene products required for both activation & resolution of inflammation. We describe the de-convolution of large-scale genome datasets to describe the components of key innate immune signaling pathways. Using a combination of computational predictions of novel protein domain structures in these datasets & cell biology validation of variant proteins in appropriate cellular contexts, we demonstrate the widespread control of inflammatory signalling pathways by inflammatory supressor molecules. Our data demonstrates the application of systematic genomic analysis to the understanding of inflammation and inflammatory disease.

Lay Abstract

IDENTIFICATION OF NOVEL REPRESSORS OF INFLAMMATION

Chronic inflammation is a serious clinical, social and financial burden on the Australian community, affecting millions of Australians from all age-groups and walks of life. A healthy immune system requires both activation and resolution of inflammatory pathways. We are studying the network of events engaged by macrophages - important cellular mediators of inflammation - in response to early inflammatory events, in an effort to identify the molecular mechanisms normally engaged to switch-off inflammation.

Dr Mai H Tran

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Scientific Abstract

Tetraspanins – new vaccine candidates for schistosomiasis

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Schistosomes are parasitic blood flukes that infect approximately 200 million people in over 74 developing countries and cause an estimated 200,000 deaths per year. Adult schistosomes adapt to the host defence mechanisms and survive for many years despite strong immune responses. The symptoms of schistosomiasis are due to the formation of granuloma and chronic inflammatory reactions that is stimulated by the eggs trapped in the liver and intestine (*Schistosoma mansoni* and *Schistosoma japonicum*) or in the bladder (*Schistosoma haematobium*).

Current control and prevention programs are based on environmental sanitation, education and treatment with the cost-effective drug, Praziquantel, however it does not prevent re-infection and drug-resistance is of major concern. Since schistosomes do not replicate in humans, a vaccine that can induce reduction in worm and egg burden, parasite transmission and fecundity would successfully prevent schistosomiasis, as opposed to vaccines for other diseases where sterile immunity is required. One of the major goals of our laboratory is to develop a protective vaccine against schistosomiasis.

In a recent manuscript (Nat. Med. 2006: *12*, 835-840), we reported two tetraspanin integral membrane proteins, *Sm*-TSP-1 and *Sm*-TSP-2, expressed exclusively on the outer tegument of adult *S. mansoni*. Tetraspanins (TSPs) consist of four conserved transmembrane regions, a small extracellular loop (EC1) and a large extracellular loop (EC2). We expressed the EC2 loop of *Sm*-TSP-1 and *Sm*-TSP-2 in *E.coli* as thioredoxin fusions and the soluble recombinant protein was then purified by affinity chromatography. To determine if these new antigens are efficacious vaccine candidates, we immunised mice subcutaneously with recombinant *Sm*-TSP-1 and *Sm*-TSP-2 emulsified with Freund's adjuvant and then challenged with *S.mansoni* infection. Worm burdens in mice vaccinated with *Sm*-TSP-1 and *Sm*-TSP-2 were reduced by 34% and 57%, respectively compared to the control group. The liver egg burden was

decreased by 52% and 64% for *Sm*-TSP-1 and *Sm*-TSP-2, respectively, and fecal egg counts were reduced by 65% in both test groups, thereby restricting transmission of the infection.

In addition, we investigated whether individuals living in schistosomiasis endemic areas generated an antibody response to *Sm*-TSP-1 and *Sm*-TSP-2. Significantly increased concentrations of human IgG_1 and IgG_3 antibodies against recombinant *Sm*-TSP-2, but not *Sm*-TSP-1, were detected in sera of individuals deemed putatively resistant to *S.mansoni* in comparison to sera from chronically infected people or control sera. Interestingly, IgG_4 and IgE antibodies, which are often associated with helminth infections, were not detected against *Sm*-TSP-2.

The high level of protective immunity shown in laboratory mice combined with the presence of anti-TSP2 antibodies found in endemic human sera suggests *Sm*-TSP-2 could be developed into a human schistosomiasis vaccine. We have now expressed the EC2 of *Sm*-TSP-2 in yeast and *E.coli*, and our aim is to improve the efficacy of the *Sm*-TSP-2 vaccine by exploring various parameters, including dosage of antigen in the presence of different adjuvants formulations, particularly those that are approved for human use. In addition, we hypothesize that tetraspanins acquire host molecules and adsorb them onto the tegument to provide an immunological mask and will determine the biological functions of TSPs in the tegument of schistosomes.

Lay Abstract

TETRASPANINS – NEW VACCINE CANDIDATES FOR SCHISTOSOMIASIS

Schistosomiasis is caused by the flatworm, schistosoma, which lives in the bloodstream of infected individuals. As the second major parasitic disease in the world after malaria, an estimated 200 million people in over 74 developing countries suffer from this severe debilitating disease. Despite wide-spread treatment with safe and effective chemotherapeutic drugs, schistosomiasis continues to be a disease of endemic proportion. Development of a vaccine would be a valuable tool to complement existing disease control and prevention programs.

Our laboratory has identified novel proteins called tetraspanins (TSPs) which elicit very high levels of protection (reduction in worm burdens and egg counts) in laboratory mice infected with schistosomes. In addition, and perhaps most encouragingly from a vaccine development perspective, antibodies were detected against TSPs in sera of individuals who are naturally resistant to schistosomiasis but not in sera from chronically infected people or unexposed donors. TSPs are expressed on the surface membrane of the schistosome, leading to the hypothesis that tetraspanins interact with molecules in the bloodstream of infected individuals. This, combined with the high protection seen in laboratory studies, suggests that tetraspanins could be developed into a human schistosomiasis vaccine.
To broaden our current knowledge of tetraspanins, we plan to investigate their functions in the schistosome and also conduct more vaccine trials in mouse model of schistosomiasis to determine the efficacy of TSPs as a schistosomiasis vaccine candidate.

Dr Stuart Macgregor

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Scientific Abstract

COST EFFECTIVE GENOME WIDE ASSOCIATION ANALYSIS

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Many complex traits and common diseases have a substantial genetic component. Major efforts have been made to identify the loci underlying this component. Identification of such loci is one of the primary means for increasing our understanding of the biochemical and developmental pathways involved in each disease. It is now clear that genome wide association (GWA) is a critical tool for effective identification of disease susceptibility loci, with several recent GWA studies published in *Nature* and *Science*. However, the major limiting factor in many GWA studies is cost. Individually genotyping the required samples (i.e. 1000s of cases/controls for hundreds of thousands of markers) for GWA is often prohibitively expensive - typical cost is >$1 million . Alternative approaches which reduce the genotyping cost are therefore highly desirable.

An approach which promises to reduce the cost of GWA is DNA pooling. In pooling, instead of individually genotyping every person in the sample, the sample is genotyped in pools of individuals. To genotype large numbers of markers, arrays are used. Tests of genetic association can be simply constructed by comparing pools but we recently showed that ignoring pooling error leads to false positives. If the pooling error can be estimated, a statistical correction can be applied. Previously, this error variance was estimated by creating replicate pools but such an approach is inefficient. Instead, we recently proposed taking advantage of the fact that there is information available across multiple markers on each array. By using this information, the pooling error variance can be estimated without recourse to multiple pools, minimizing cost. Best results were obtained by using a statistical model based on a general linear mixed model (SM publication 18). Recently I further extended the method, giving guidelines for best study design (SM publication 23).

With the above statistical framework, we applied pooling to melanoma and endometriosis, with a similar study of glaucoma underway. In the case of endometriosis, a sample of 384 cases/controls was used (3 arrays per pool). The estimates of pooling error variance were such that the amount of information (relative effective sample size compared with individual genotyping) extracted from the pooling experiment was ~80%. Taking into account the reduced number of arrays used with pooling, the cost saving compared with individual genotyping was >100 fold. This meant the cost of our endometriosis GWA was <$10000. Performing the same study with individual genotyping would have cost >$0.5 million. ~100 markers from pooling were individually genotyped. Concordance between pooling and individual genotyping was excellent. The vast majority (>96%) of markers associated in pools

were also associated ($p<0.05$) with individual genotyping. We are currently following up these results with further genotyping in independent endometriosis samples. Our pooling based GWA melanoma study provided similarly encouraging results and further genotyping is planned. New applications of my novel approach are set to include pooling based GWA analysis of alcohol/nicotine dependence and corneal thickness (subject to funding), as well as analysis of pooling data supplied by US company JK Autoimmunity.

Lay Abstract

COST EFFECTIVE GENOME WIDE ASSOCIATION ANALYSIS

Genetic studies over the last 20 years have increased our understanding of the underlying biology for a range of diseases. Previously, successful studies utilised family-based samples (using sparse sets of genetic markers). New technologies now allow denser sets of markers, facilitating the use of a powerful population-based study design (genome wide association or GWA). However, this is often prohibitively expensive. The cost can be reduced by using DNA "pooling". We have shown that advanced statistical methods are required for analysis and interpretation of pooling data. Coupled with our new statistical method, DNA pooling offers up to 100-fold reduction in cost compared with alternatives.
We have applied our novel statistical method to data on melanoma and endometriosis. Melanoma is a form of skin cancer which underlies the majority of skin cancer related deaths. Endometriosis affects up to ten percent of women and is associated with pelvic pain and infertility. By applying pooling techniques to large samples of disease cases and controls we have been able to identify genes contributing to disease susceptibility in melanoma and endometriosis. Knowledge of the relevant genetic variants will enable us to define pathways leading to disease.

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Scientific Abstract

AN ENU SCREEN IN THE MOUSE REVEALS A ROLE FOR WSTF IN WILLIAMS SYNDROME

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Epigenetic modifications to the genome are crucial for the correct regulation of transcription, are mitotically stable, and are essential for differentiation of cell lineages, and the fidelity of cell type within lineages. Proteins involved in the establishment and maintenance of the epigenetic state include DNA methyltransferases (e.g. Dnmt1, Dnmt3a), which methylate cytosine residues in the DNA, histone modification enzymes (e.g. histone deacetylases and methylases), which catalyse modifications to the histone proteins, and chromatin remodelling proteins (e.g. SWI/SNF complex proteins). Some human diseases have been linked to mutations in genes that encode epigenetic modifiers. For example, Rhett syndrome, a disease associated with early-onset mental retardation, results from mutations in the methyl-binding protein MeCP2, and mutations in ATRX, a SNF-2-like chromatin remodelling protein are associated with mental retardation, α-thalassaemia and other developmental abnormalities.

We have used a sensitised ENU mutagenesis screen to identify mouse mutants displaying altered epigenetic processes. Our screen relies on an epigenetically-sensitive, red blood cell specific GFP transgene that is expressed in approximately 60% of erythrocytes in the FVB inbred mouse strain. To date we have identified fifteen mutant lines from our screen. Linkage analysis reveals that the mutations map to unique chromosomal locations. We have identified the genes underlying six of the mutations, *SmchD1*, *Dnmt1*, *Snf2h, Foxo3a*, *Williams Syndrome Transcription Factor* and *Uble1b*.

The human homologue of *Williams Syndrome Transcription Factor* (*WSTF*) lies in the Williams Syndrome linked region. This syndrome affects approximately 1 in 20,000 live births, and while generally not fatal, the risk of unexpected death is 25-100 fold higher than normal. It is a pleiotropic disease characterised by cardiovascular defects, elfin-like facial features, mental retardation, short stature and other developmental abnormalities. There are approximately 28 genes in the linked interval (including *elastin*, *GTF2IRD1* and *WSTF*), and there is considerable debate about the role of each of these genes in the disease phenotype. There is strong evidence that hemizygosity for the elastin gene is causative of some of the cardiovascular phenotypes, and some evidence that *GTF2IRD* plays a role in the craniofacial abnormalities. Through our ENU mutagenesis screen, we have created mice with a hypomorphic allele of WSTF. This is the first mouse to be made carrying a mutation in this

gene. Our *WSTF* mutant homozygous mice show reduced survival, are significantly smaller than littermates and have abnormal craniofacial features reminiscent of those seen in Williams Syndrome individuals. Our results suggest that WSTF plays a role in the craniofacial phenotype of Williams Syndrome, and provides a tool to increase our understanding of craniofacial development.

Lay abstract

THE IDENTIFICATION OF A NEW GENE INVOLVED IN CRANIOFACIAL DEVELOPMENT

Williams Syndrome affects approximately 1 in 20,000 people world-wide. People with this disease generally display a characteristic elfin-like face, and a distinctive, overly social personality, combined with mental retardation, and heart defects. There are approximately 28 genes that lie in a region of Chromosome 7 known to be deleted in individuals with Williams Syndrome, but the role of each of these genes with respect to the symptoms has not yet been fully elucidated. We have created a mouse with a mutation in one of these genes, *Williams Syndrome Transcription Factor*. This is the first time such a mouse has been created. Mice carrying this mutation exhibit facial defects reminiscent of those seen in people with Williams Syndrome, suggesting at least some of the facial abnormalities may be caused by the loss of WSTF gene. The mutant mouse provides us with a model to improve our understanding of the molecular basis of this disease.

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Scientific Abstract

GENE THERAPY FOR GLIOBLASTOMA MULTIFORME: A NOVEL TREATMENT FOR A FATAL DISEASE

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BACKGROUND: Glioblastoma multiforme (GBM) is the commonest primary brain tumour and remains a neurosurgical and oncological enigma. Extirpation followed by an array of both medical and radiation regimens over the years has done little to change the prognosis for patients with this tumour who usually succumb to the disease within 1 year. Hence, a novel therapeutic modality is required if the survival of patients with this disease is to be improved.

The ATM gene, which is mutated in the disease ataxia-telangiectasia (A-T), is implicated in response to radiation-induced DNA damage, leading to profound radiosensitivity. By reducing the levels of ATM in the radioresistant GBM cells through RNA interference (RNAi), the tumour can be transformed from radioresistant to radiosensitive. Concurrently, advances in science have demonstrated that the lentivirus is the most effective method of delivering genes into quiescent cells. By producing safe non-replicating lentiviruses, containing RNAi ATM, GBM can be sensitised to radiotherapy. In conjunction with surgery, this strategy will provide an enhanced therapeutic intervention especially in the case of GBM where the tumour is untreatable.

AIM: To sensitise GBM tumour cells to radiation by decreasing/aborting the function of ATM in these cells using lentiviral-mediated RNAi gene transfer and thus enhancing radiotherapeutic efficiency.

METHODS: Lentiviruses, which contain RNAi ATM, are produced in high titre and GBM cells are infected to confer radiosensitivity.

RESULTS: RNAi ATM has been successfully cloned into the lentiviral vector and lentiviruses expressing RNAi ATM have been successfully developed. GBM cells are infected at nearly 100% efficiency and infected GBM cells demonstrated ATM protein reduction by more than 90% of normal levels, reduced ATM s1981 phosphorylation and foci formation, reduced p53-s15 phosphorylation and at least 3-fold radiosensitisation.

CONCLUSION: Success in this approach will provide a novel and exciting strategy for the treatment of GBM and thus improving the survival of patients with these tumours.

Lay Abstract

GENE THERAPY FOR GLIOBLASTOMA MULTIFORME: A NOVEL TREATMENT FOR A FATAL DISEASE

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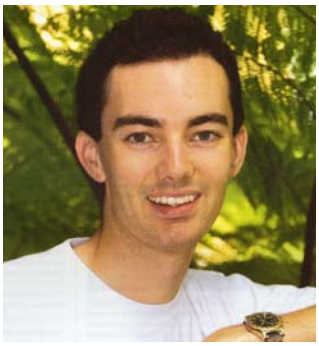
BACKGROUND: Glioblastoma multiforme (GBM) is the commonest primary brain tumour of which death is due to failure to control the growth of the tumour. Effective therapies for GBM remain elusive and even with surgery, radiation treatment and chemotherapy, patients succumb to this disease within one year. Hence, a novel therapeutic modality is required if the survival of patients with this disease is to be improved. ATM gene mutation in the disease ataxia-telangiectasia (A-T) leads to profound radiosensitivity. By reducing the levels of ATM in the radioresistant GBM cells through RNA interference (RNAi), the tumour can be transformed from radioresistant to radiosensitive. Concurrently, advances in science have demonstrated that the lentivirus is the most effective method of delivering genes into quiescent cells. By producing safe non-replicating lentiviruses, containing RNAi ATM, GBM can be sensitised to radiotherapy. In conjunction with surgery, this strategy will provide an enhanced therapeutic intervention especially in the case of GBM where the tumour is untreatable. *AIM:* To sensitise GBM tumour cells to radiation by decreasing/aborting the function of ATM in these cells by gene therapy. *RESULTS:* ATM knockout lentiviruses have been successfully produced. GBM tumour cells are infected at nearly 100% efficiency and infected GBM cells demonstrated ATM protein reduction by more than 90% of normal levels, reduction in the ATM s1981 and p53 gene function and at least 3-fold radiosensitisation. *CONCLUSION:* Success in this approach will provide a novel and exciting strategy for the treatment of GBM and thus improving the survival of patients with these tumours.

Mr Stephen Earl
The Queensland Institute of Medical Research



Scientific Abstract

ANALYSIS OF AUSTRALIAN SNAKE VENOMS FOR THE DISCOVERY AND DEVELOPMENT OF NEW HUMAN THERAPEUTICS

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Australian snake venoms are potent cocktails of bioactive proteins that interfere with several mammalian physiological processes. In many cases, these venom proteins have an extremely stable structure, are very specific in their site of action and act with high efficacy. Also, when compared to exotic snake venoms, Australian snake venom composition has not been extensively studied and thus these venoms likely contain as yet undiscovered components. For these reasons, we hypothesise that Australian snake venoms are a potential source of new and improved human pharmaceuticals, because despite significant medical advances over the last few decades, a number of serious health conditions including pain, cancer and stroke remain without adequate pharmaceutical treatments. Hence, pharmaceutical development to treat these conditions is urgently needed and is of great significance to improved health and medical care.

This project has employed a combined approach of transcriptomics through a venom gland cDNA microarray and proteomics via 2-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS) to systematically examine the venoms of several Australian elapid snakes to identify all venom components and from these select novel venom proteins with potential for development as new human therapeutics. The venom gland cDNA microarray employed expression profiling between the venom gland and the liver to identify venom gland specific transcripts. From these, a number of previously known venom proteins were identified, along with new venom proteins and two potential therapeutic candidates. When separated by 2-DE, the Australian venoms showed approximately 100-200 discrete protein spots, varying in molecular weight from 7 to over 100 kDa and pI from 3 to 10. Using MS, approximately 80 percent of protein spots have been identified. These include previously characterised venom proteins such as phospholipase A_2 enzymes, neurotoxins and prothrombin-activating proteins. A number of novel venom proteins have also been identified from the proteomic work and

three of these have been selected as potential therapeutic candidates. Further transcriptomic strategies such as RACE and RT-PCR have successfully been employed to obtain the nucleotide coding sequence for these candidates from venom gland cDNA. This has enabled recombinant expression of these proteins for functional characterisation. Initial functional assays have proven successful for two of the therapeutic candidates investigated so far. Future work will now focus on further expression and characterisation of candidate proteins and fully evaluating their therapeutic potential.

Lay Abstract

DEVELOPING NEW HUMAN MEDICINES FROM AUSTRALIAN SNAKE VENOMS

Australian snake venoms are potent protein cocktails that contain a number of bioactive molecules. Despite being toxic as a mixture, individual venom proteins can have beneficial effects when purified and when used in controlled doses. These include molecules which may have application in blood disorders, pain and wound healing. Hence, snake venoms are a potential source of new human medicines. Despite major medical advances in recent decades, a number of serious health conditions such as cancer, stroke and pain remain without adequate pharmaceutical treatments and thus new drugs against these disorders are urgently needed. This project has undertaken a systematic study of several Australian snake venoms to identify all venom components and also novel venom proteins with potential for development as new human medicines. We have used a combined approach of searching for new genes corresponding to new venom proteins and also looking at the venoms themselves in a global fashion to identify all venom proteins. From this work, a number of previously known venom proteins have been identified along with new venom proteins and several of these have been selected as potential drug candidates. Initial functional testing for two candidates has provided promising data. Future work will now focus on further characterisation of candidate proteins and fully evaluating their therapeutic potential.

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Scientific Abstract

The role of ultraviolet radiation in molecular pathways to melanoma

Australia has the highest rate of melanoma in the world (Australian bureau of statistics, 2005). Sun exposure is a major risk factor for developing this disease. We have been studying ultraviolet radiation (UVR)-induced malignant melanoma (MM) development in mice carrying Ink4a/cdk4/pRb and Ras/Raf/MAPK pathway defects. The animals we have used carry either a melanocyte-specific mutant Hras (G12V) transgene (TPras), an oncogenic mutation (R24C) in Cdk4, or a combination of the two. Brown TPras or TPras/Cdk4R24C/R24C mice (mixed C3H/Sv129 background) were treated with a single neonatal UVR dose. Pups (2-3 days old) were exposed to a dose of 8.15 kJm (UVB 280-320 nm). The UVR-treated cohorts of TPras and TPras/Cdk4R24C/R24C mice were studied for MM development over a period of 1 year. It has previously been shown that adult TPras mice do not develop MM when treated with chronic doses of UVR (5.6-8.06kJ/m2 biweekly for 28 weeks). However, after a single neonatal UVR treatment we found that the MM incidence increased to 57% at 1 year. These results echo the findings of epidemiologists that show childhood sun exposures hold the greatest risk to developing melanoma latter in life.

Cdk4R24C/R24C/TPras mice developed MM spontaneously with a penetrance of 58%, which rose to 83% after neonatal UVR. By comparing UVR-induced tumours with those not related to UVR exposure we identified a genomic signature. This signature was further validated in a set of human MM (n=147), comparing MMs from areas of chronic sun exposure (head) and intermitted sun exposure (trunk). This genetic signature provides an insight into the role UVR plays in the development of melanoma. If the role of UVR was better characterized, improved strategies for preventative medicine could be applied, and mortality reduced. Future applications could see the development of post-sunburn applications that would suppress tumour formation.

All TPras lesions were small in situ cutaneous melanomas, while 92% of Cdk4R24C/R24C/TPras animals that developed melanoma had metastatic tumours. In this model Ras activation alone is sufficient to predispose melanocytes to UVR-induced transformation, with mutant Cdk4 more important for tumour progression, producing larger more aggressive, metastatic MMs. The molecular differences were explored between the MMs from mice of different genotypes by overlaying expression and array CGH data. Several genes were identified that showed co-ordinate gene/copy number and expression changes, some of which were further validated in human MM using tissue arrays. Melanomas often metastasize early and are generally intractable to current therapeutic regimens. By identifying genes involved in tumour progression alternative drug therapies can be developed to reduce mortality rates.

Lay Abstract

The role of ultraviolet radiation in molecular pathways to melanoma

Several genes are known to be involved in the development and progression of melanoma and UV light has also been identified to play an important role in this process. This study found that mice with mutations in melanoma susceptibility genes that received a single neonatal dose of UV radiation (UVR) developed melanoma over the course of 1 year. Adult mice of this strain do not develop melanoma when treated with chronic doses of UVR. These results echo the findings of epidemiologists who have shown that childhood sun exposures hold the greatest risk to developing melanoma later in life. This work also found that mice with 2 melanoma susceptibility genes mutated developed melanoma spontaneously with a penetrance of 58%, which rose to 83% after neonatal UVR. By comparing UVR-induced tumours with those not related to UVR exposure we identified a genomic signature of UVR treatment. This signature was further validated by comparing human melanoma (n=147) from areas of chronic sun exposure (head) and intermitted sun exposure (trunk). If the precise role of UVR in this disease could be better characterized, improved strategies for preventative medicine could then be applied. Future applications could see the development of post-sunburn applications that would suppress future tumour formation. By studying the key molecular events that underlie UVR-induced melanoma we hope to understand why some people are more susceptible to melanoma than others, and help us better manage our lives in the Queensland sun.

Dr Susan Jordan

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Scientific abstract.

DETERMINANTS OF SEROUS OVARIAN, FALLOPIAN TUBE, AND PERITONEAL CANCERS: A NEW PERSPECTIVE

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Background: Ovarian cancer affects approximately 1500 women in Australia each year. Because it is often diagnosed at an advanced stage, the prognosis is poor, particularly for invasive serous cancer, the most common subtype. At this time there are no effective screening strategies to facilitate early diagnosis, partly because so little is known about how these cancers develop. Serous cancers also occur in the peritoneum and fallopian tube although they are diagnosed much less frequently than ovarian cancer and very little is known about their causes. Traditionally, serous cancers of the ovary, peritoneum and fallopian tube have been classified as separate diseases, however given their close histological and clinical similarities, all three may be variants of the same malignancy. If this were the case it would have implications for our understanding of both the cellular origins of the cancers, and the processes involved in their development. A comparison of risk factors for serous ovarian, peritoneal and fallopian tube cancers will increase our understanding of the relationship between the three cancer types and may shed some light how serous ovarian cancers develop.
Methods: We investigated risk factors for the three cancers using data from a large Australian population-based case-control study. We included women with incident invasive serous ovarian (n=627), primary peritoneal (n=129) and fallopian tube (n=45) cancer and 1508 control women. Participants completed a comprehensive reproductive and lifestyle questionnaire. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs).
Results: Hormonal contraceptive use was inversely related to risk of all three cancers with OR for 5+ years vs never use of 0.5 (95%CI 0.4-0.6) for ovarian, 0.5 (0.2-1.1) for fallopian tube and 0.7 (0.4-1.1) for peritoneal cancer. Parity and breast-feeding were also inversely related to risk of serous ovarian and fallopian tube cancer. In contrast, parous women had an increased risk of peritoneal cancer (OR=1.8, 95%CI 0.8-3.9), and increasing parity did not lower risk. There was also no association between breast-feeding and peritoneal cancer.

However, obesity was associated with a doubling of risk for peritoneal cancer alone (OR=2.3, 95%CI =1.4-3.7).
Conclusion: The very similar patterns of risk for serous ovarian and fallopian tube cancers suggest they might develop in a similar way. One clear possibility is that both arise in the fallopian tube which might explain why a defined precursor lesion has not been found for serous ovarian cancer. The somewhat different results for primary peritoneal cancer suggest that peritoneal cancers may develop along a separate pathway. Also since the fallopian tube epithelium is physically unaffected by ovulation, these results also call into question the widely accepted role of ovulation in the development of serous ovarian cancer. They suggest instead that the hormonal and/or chemical effects of ovulation are more important.

Lay Abstract

DETERMINANTS OF SEROUS OVARIAN, FALLOPIAN TUBE, AND PERITONEAL CANCERS: A NEW PERSPECTIVE

Ovarian cancer affects almost 1500 women in Australia every year. Because it is often diagnosed after it has spread, the prognosis is poor, particularly for women with serous cancer, the most common type. There are no effective screening programs to detect the cancer early, partly because so little is known about how these cancers develop. Serous-type cancers also occur in the lining of the pelvis (peritoneum) and the fallopian tubes, although they are not as common as ovarian cancer and very little is known about their causes. Traditionally, ovarian, peritoneal and fallopian tube cancers have been classified as separate diseases, however they are microscopically similar and are treated in the same way, so in reality all three may be variants of the same cancer. If this were the case it would have implications for our understanding of how they develop. Comparison of the risk factors for serous ovarian, peritoneal and fallopian tube cancers will shed some light how serous ovarian cancers develop. In this study women with ovarian, fallopian tube and primary peritoneal cancer were compared to women without cancer. We found that the taking the contraceptive pill, breast feeding, and having several pregnancies protected against both fallopian tube cancer and serous ovarian cancer. Having pregnancies and breast-feeding did not protect against peritoneal cancer however, but obesity was associated with an increase in the risk of only this type of cancer. These findings add to evidence suggesting that serous ovarian cancers might actually arise in the fallopian tube but suggest that peritoneal cancers may develop along a different pathway. This information may help with the development of new methods of screening for ovarian cancer and also provides new insights into the causes of fallopian tube and peritoneal cancers.

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Scientific Abstract

DETERMINING A NOVEL MECHANISM FOR GROWTH HORMONE RECEPTOR DIMERIZATION AND ACTIVATION

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Growth Hormone (GH) is a major regulator of postnatal growth, metabolism, fertility cellular proliferation and differentiation. GH is implicated in a number of disease states including dwarfism, giantism and cancers. Because of these widespread clinical applications for GH, there has been considerable interest in the mechanisms of action of GH and its receptor. It was originally thought that GH initiated its actions by sequentially binding two GH receptor (GHR) monomers causing dimerization and initiating the intracellular signalling cascades, including JAK2, STAT5 and MAPK.

However, recent evidence by our group and others suggested dimerization alone was insufficient for GHR activation. Therefore, the aims of this current study were to determine if the GHR exists as constitutive dimers in living cells and examine the region of the receptor responsible for dimerization. To do this Fluorescence Resonance Energy Transfer (FRET) was employed. FRET, a biophysical technique to determine protein-protein interaction, provided clear evidence for constitutive dimerization of the human (h) GHR in living cells (Brown et al., 2005, Nat. Struct. Mol. Biol. *12*, 814-821). FRET studies also showed the extent of dimerization is unaffected by hGH. Finally, using FRET constructs truncated at the intracellular and/or extracellular domains (ICD and ECD) of the hGHR, it was shown that the transmembrane domain (TMD) is required for stabilizing constitutive hGHR dimerization.

The ToxR System, a bacterial assay for examining TMD interactions, was then used to independently confirm hGHR TMD interactions. It was found that constructs consisting of various parts of the hGHR TMD produced robust β-galactosidase signal, indicating interaction along the length of the TMD. A number of point mutations were able to decrease or increase activity, inferring certain amino acids may be necessary or detrimental to dimerization.

Finally, an approach to determine the active or inactive orientation of the hGHR TMD was carried out. This involved removing the GHR ECD and fusing a coiled-coil dimerization domain directly to the helical GHR TMD. The transmembrane and intracellular domains were sequentially rotated by the insertion leucine residues. This led to 2 constructs being able to constitutively activate JAK2 and STAT5 signalling molecules, while 2 orientations of the

helix were not signalling competent. Finally, computer modelling allowed the determination of the residues in each of the active and inactive orientations.

Taken together, these results support a model for hGHR activation by rotation of preassociated receptors, rather than by hormone-induced dimerization of two monomers. This new mechanism for hGHR activation will facilitate the future rational design of GH mimetics for use in cancer treatments and tissue regeneration.

Lay Abstract

A NOVEL MECHANISM OF GROWTH HORMONE RECEPTOR ACTIVATION

Growth hormone is one of the most important hormones in the body and is responsible for overall body growth, fertility, muscle and fat metabolism. Mutations in Growth Hormone or its receptor cause diseases including dwarfism, giantism, and cancers including lymphoma, colorectal and breast cancer. The Growth Hormone and its receptor can also be exploited for many novel therapies including to assist skin repair after burns, to assist the body to self renew and slow the aging process, increase muscle mass in wasting disorders, decrease fat deposition in the obese and diabetic related complications. Unfortunately, as GH is a large protein, it can only be administered via injection so currently, a major focus in drug discovery is to make a Growth Hormone-like drug that could be taken as a pill. Because of the myriad of potential uses for Growth Hormone in treating many disorders listed above, it is extremely important to uncover the exact mechanism GH activates the Growth Hormone Receptor, to open the door for Growth Hormone to be more widely used in clinical practice. This current study used a variety of conventional and novel techniques to discover a novel mechanism for Growth Hormone Receptor activation that will revolutionise the future design of Growth Hormone like drugs, as well as the understanding of related hormone receptors.