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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.

Dr Teong Chuah
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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

Dr Teong Chuah
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Department of Surgery, University of Queensland

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.

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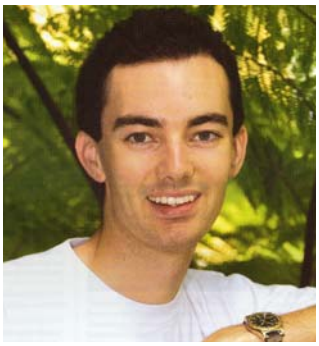

Scientific Abstract

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.