Post-graduate Student Award Finalists

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Institute for Molecular Bioscience, University of Queensland
Research Funding: NIH Grant

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Research Funding: NHMRC Grant

Daniel Lee Timms
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Research Funding: Prince Charles Hospital

Post-doctoral Award Finalists

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Research Funding: NHMRC

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Research Funding: NHMRC Grant

Senior Post-Doctoral Award Finalists

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Research Funding: NHMRC, Roche Australia and ACITHN (Australian Centre for International Tropical Health and Nutrition)

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Research Funding: ARC Grant

Grant A. Challen

IN SEARCH OF KIDNEY STEM CELLS

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ABSTRACT:

In Australia, there are over 60,000 cases of advanced CRD with about 7000 ESRD patients receiving dialysis at a total cost of AU$360 million per annum. The current treatment options are dialysis, which is expensive and has considerable morbidity, and organ transplantation which is limited by the number of suitable donor organs available for transplant. Alternative treatment strategies are continually being sought and one option currently being explored is the potential for stem cell therapy to either repair or supplement the function of kidneys in renal failure patients. The main goal of this study was to attempt to identify and isolate potential stem cell populations from embryonic and adult mouse kidneys. Two approaches have been undertaken in this thesis to facilitate this; (1) determining the molecular phenotype of the renal progenitor population via microarray expression profiling and (2) the characterisation of purified potential stem cell populations from embryonic and adult mouse kidneys.

(1) We have constructed a comprehensive gene expression profile of the initial stages of kidney development including for the first time a direct analysis of the crucial timepoint where the renal progenitor population is formed. By spatially comparing E10.5 uninduced metanephric mesenchyme (the renal progenitor population) to rostral intermediate mesoderm and temporally to later crucial stages of murine kidney development, a suite of genes that define the renal progenitor population have been identified and their expression patterns analysed throughout embryonic renal development via *in situ* hybridisation. Using bioinformatics to define the genes that encode for transmembrane domain proteins, we have sought to identify cell surface markers of the renal progenitor population that might maintain expression in the adult kidney and mark a residual renal stem cell potential. These markers, such as CD24a antigen and cadherin-11, are being used to purify cells with renal progenitor cell characteristics from developing and mature kidneys by fluorescence-activated cell sorting (FACS).

(2) We have characterised renal side population (SP) at the molecular and functional level. SP cells are isolated by FACS based on their ability to efflux the vital dye Hoechst 33342 and SP cells from numerous organs have been shown to contain a stem cell population. The SP fraction of embryonic (E15.5) and adult mouse kidneys was found to represent 0.1-0.2% of the total viable cell population and immunophenotyping of these cells shows they are phenotypically quite distinct from bone marrow SP cells, lacking expression of markers such as CD45, CD34 and c-kit, suggesting the SP found in these kidneys is not of haematopoietic origin. By Affymetrix microarray analysis, we have identified common genes expressed between bone marrow, embryonic kidney and adult kidney SP cells that may represent a putative SP molecular signature. By analysing the expression patterns of these genes by wholemount and section *in situ* hybridisation, we hope to identify the *in vivo* niche of these cells. There are relatively few differences in gene expression profiles between embryonic and adult SP cells, indicating the SP phenotype is conserved throughout kidney development. The functional capacity of renal SP cells have been tested in various ways. Microinjection of adult kidney SP cells into stage 4 chick embryos showed they are capable of engraftment into mesonephric tubules and mesenchymal cells of the developing metanephros *in ovo*. Engraftment of adult kidney SP cells into embryonic (E12.5) mouse kidneys has shown that they are capable of contributing to collecting tubules and developing nephrons and the engraftment rate of SP cells compared to total kidney cells was increased 13-fold. These models together with traditional *in vitro* differentiation assays and *in vivo* mouse models of renal disease are now being employed to test the ability of various kidney cell populations to act as renal stem cells.

Jennifer A. Kricker

Functional analysis of the impact of glycosylation and heparin-binding regions of IGFBPs on the interaction of IGF-I with vitronectin

Kricker JA, Herington AC, Upton Z.

Previous studies demonstrated that IGF-II binds directly to vitronectin (VN) while IGF-I binds poorly (Upton *et al*, 1999). However, binding of VN to integrins has been demonstrated to be essential for a range of IGF-I-stimulated biological effects including IGF binding protein-5 (IGFBP-5) production, IGF type-1 receptor autophosphorylation and cell migration (Jones *et al*., 1996). Thus, this PhD research project examined the hypothesis that a link between IGF-I and VN must occur and may be mediated through IGFBPs. Studies using competitive binding assays with VN and [^{125}I]-labelled IGFs in the absence and presence of IGFBPs revealed IGFBP-4, IGFBP-5 and non-glycoyslated IGFBP-3 significantly enhance binding of IGF-I to VN, while IGFBP-2 and glycosylated IGFBP-3 had a smaller effect. Furthermore, binding studies with analogues indicate that glycosylation status of IGFBP-3 and the heparin-binding domains of IGFBP-3 and IGFBP-5 are important in this interaction. The functional significance of IGFs binding to VN on cell migration in MCF-7 breast carcinoma cells was examined and cell migration was found to be enhanced when VN was pre-bound to IGF-I in the presence of IGFBP-3, -4 and -5. The effect required IGF:IGFBP:VN complex formation; this was demonstrated by use of a non-IGFBP-binding analogue, des(1-3)IGF-I. Comparison of various IGFBP-3 preparations, produced in different expression systems or with select mutation of glycosylation sites, demonstrated that glycosylation affects cell migration in response to IGF-I:IGFBP:VN complexes. Thus, we found higher levels of cell migration with the less glycosylated proteins. Findings with complexes containing heparin-binding domain mutants of IGFBP-3 and -5 compared to their wild-type proteins, revealed there was little difference between the mutant and wild-type complexes on their ability to stimulate MCF-7 cells to migrate. Additionally, heparin had no effect on IGFBP-5 complex-stimulated migration. However, heparin was able to reduce IGFBP-4 complex-stimulated migration. These findings taken together with earlier data have led us to propose a new model by which IGFBP-3 and -5 mediate IGF-I binding to VN, and that this interaction differs from IGFBP-4-mediated IGF-I binding. This data also suggests that glycosylation status of IGFBP-3 may regulate breast cancer cell migration in the presence of IGF-I and VN. Together, these data indicate the importance of IGFBPs in modulating IGF-I binding to VN and that this binding has functional consequences in cells. Future directions for this work include investigations into the mechanisms underlying formation of the trimeric complex and the associated signalling pathways involved.

This work led to the filing of an international patent (WO 02/24219A1), which forms the critical part of the platform technology, VitroGro®, that is being commercialised by Tissue Therapies Ltd. The technology is being explored in a range of biomedical applications in which the cell growth and migration is required, including: the replacement of serum for the culture of cells for the production of pharmaceuticals and generation of cell-based therapies; wound healing; and coating implants and devices.

Daniel Lee Timms

Scientific Abstract

A Novel Artificial Heart to Alleviate the Donor Shortage for Heart Transplantation Surgery

Heart disease is the world's biggest killer, with 800 000 new patients each year diagnosed with heart failure worldwide. However, there is a shortage of donor hearts available to patients requiring heart transplantation, as advocated by the David Hookes Foundation. In fact, just 3000 heart transplant operations are performed each year. This limited supply of donor organs has accelerated the demand for the development of an artificial mechanical alternative.

Studies have revealed that patients receiving mechanical devices to assist a failing left ventricle have extended lives and improved quality of life compared to recipients of drug therapy. However, these patients succumb to various ailments associated with the current commercially available pulsatile type devices inside two years.

There are two types of new cardiac assist devices currently under development: Left Ventricular Assist Devices (LVAD), such as Sydney Based Ventracor's "Ventrassist^{TM}", which assist a failing left ventricle only; and Right Ventricular Assist Devices (RVAD) that assist a failing right ventricle only. The thirty percent of patients suffering bi-ventricular heart failure therefore require the implantation of two devices, increasing overall system size and thus reducing the availability of this vital technology to women and children. There is currently no commercially available implantable single device system that provides assistance to both ventricles of a failing heart with one rotary pump.

Therefore, this research conducted at QUT and TPCH focused on developing a continuous flow rotary type bi-ventricular assist device (Bi-VAD) that has the capability to assist both ventricles of a failing heart simultaneously. This novel device employs magnetic and hydrodynamic suspension techniques to float the rotating impeller. These techniques reduce blood damage and component wear, thus enabling the device to potentially assist a failing heart for periods in excess of 10 years.

The novel device is the subject of Patent (PCT) Application #WO2004/098677, and follow up Provisional Patent Application #AU2004906579. The device is currently undergoing continued In-Vitro proof of concept trials, with In-Vivo clinical trials scheduled for August 2005 at TPCH. This device has been under development at QUT and TPCH since 2001, and has since been identified for future development within the Institute of Health and Biomedical Innovation (IHBI), and Medical Engineering Research Facility (MERF). Both research centers are supported by the Queensland Government Smart State Research Facilities Fund, and are designed to conduct pioneering studies in health, biomedical science and biomedical engineering, including Australia's emerging needs in orthopaedic and artificial organs research.

Successful development of this innovative Bi-Ventricular Assist device will reduce the need for patients requiring heart transplant surgery to wait for the unfortunate demise of a correctly matched donor to provide the required organ. Furthermore, those patients considered not eligible for cardiac transplantation may be provided a much needed alternative. Once the device is implanted, these patients will be awarded the flexibility to leave the confines of the hospital and return to daily activities, thus dramatically improving their extended quality of life.

Becky L. Conway-Campbell

Nuclear localization of the growth hormone receptor is associated with dysregulated cell cycle progression and tumorigenesis *in vivo*.

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In recent years it has become evident that Growth Hormone (GH) excess patients are predisposed to a variety of cancers, with colorectal, breast, and haematological malignancies being reported most frequently. Furthermore, a significant risk of metaplasia with long term GH treatment has been reported. Elevated GH receptor (GHR) expression has been shown in a range of human malignancies (lymphoma, melanoma, rhabdomyosarcoma, ovarian and breast carcinoma) and of particular note has been the presence of nuclear localized GHR in a high proportion of these specimens. Therefore, we have investigated the role of nuclear localization of the GHR in cellular proliferation and transformation.

Our work has identified that nuclear GHR is correlated with high proliferative status *in vivo*, and that nuclear-targeted GHR causes a dysregulation of proliferative arrest and induces cell cycle progression in a pre-leukemia cell line BaF-GHR. Microarray analysis of these lines compared to WT controls indicated chronic upregulation of genes for proliferation inducers (*Survivin* and *Mybbp1a*), for markers of tumorigenicity (*Cathepsin C* and the serine biosynthesis enzyme *Phgdh*), and also genes associated with metastatic potential (*Dysadherin*). Upon injection of nuclear-targeted GHR expressing cells into immunocompromised nude mice, these cells were able to form large aggressive tumours at every injection site. Maximal tumour burden was reached within 16 days, when mice were euthanased according to Australian ethical guidelines. Histological analysis indicated that the tumours were highly aggressive invading into the dermis. Metastatic potential of the tumours was also high. All lymph nodes were found to be full of tumour cells with only a small section remaining normal, and there was also evidence of tumour formation in the livers of the mice.

In conclusion, our study supports the view that GHR nuclear localization results in cellular transformation. Normal cells possess the ability to regulate nuclear localization but when this process is dysregulated, tumorigenesis can result. Since we observe nuclear localized GHR in solid tumour sections from lymphoma patients as well as normal proliferating cells, strategies aimed at blocking nuclear expression of the GHR may prove to be a useful cancer therapeutic.

Chung Fai Wong

Regulation of Squamous Differentiation By The E2F Family of Transcription Factors

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The skin is an essential organ of the body, acting as a first line of defence against dehydration, injury and infection. To maintain the integrity of the skin, keratinocytes in the basal layer of the epidermis undergo irreversible growth arrest, followed by a transcriptionally-regulated process of squamous differentiation. As the keratinocytes differentiate, they alter both biochemically and morphologically, acquiring a range of genes that are responsible for the highly cross-linked, keratinised structure of the skin. Failure of keratinocytes to undergo irreversible growth arrest or commit to squamous differentiation may result in squamous neoplasia. Squamous cell carcinomas (SCCs) have the capacity to metastasise and consequently are considered life-threatening. Current treatment options focus primarily on tumour excision for early diseases or surgery followed by chemo-radiation therapy for later disease. These therapies are, of necessity, non-specific and are associated with significant morbidity and cost. The development of novel therapies specifically targeting the disrupted proliferation and differentiation programme in SCCs would be invaluable.

In our research, we have identified a novel role for the E2F family of transcription factors in the regulation of squamous differentiation. It is accepted that E2F contributes to the proliferation of human epidermal keratinocytes (HEKs). In the present study we provide data that demonstrates that E2F also actively suppresses the commitment of keratinocytes to undergo squamous differentiation. Moreover, E2F overexpression in differentiated keratinocytes suppresses the activity of differentiation-specific marker genes indicating that E2F may also act as a modulator of the differentiation process. Taken together, these results imply that E2F overexpression in keratinocytes could contribute to deregulated proliferation of these cells and a delay in their commitment to undergo terminal squamous differentiation. Interestingly, E2F overexpression occurs in SCCs and SCCs are associated with deregulated proliferation and an inability to undergo terminal differentiation. Based on this we would predict that anti-E2F therapies could represent an effective treatment for SCCs as targeting E2F could potentially (i) stop uncontrolled proliferation of neoplastic keratinocytes and (ii) facilitate the initiation of squamous differentiation. Accordingly, when we inhibited E2F activity in a differentiation-incompetent SCC cell line (KJD-1/SV40) and subsequently treated the cells with differentiation-inducing stimuli, both the activity and expression of a range of differentiation-specific marker genes was reinstated. These data indicate that inhibition of E2F activity is able to stimulate cell cycle withdrawal and to sensitise cells to subsequent differentiation-inducing stimuli. Our results also illustrate the therapeutic potential of developing an E2F inhibitor as a novel differentiation therapy for squamous cell carcinomas.

Masa Cemazar

ABSTRACT

Bioengineered cyclic peptides for the treatment of multiple sclerosis

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Multiple sclerosis (MS) is believed to be caused by an autoimmune reaction against antigens from a number of different proteins composing the myelin sheet. Previous studies have identified a number of peptide sequences from myelin oligodendrocyte protein (MOG) that behave as MS antigens, which could bind to MS antibodies and thereby prevent the deleterious effect of demyelinating antibodies and/or initiate a protective T-cell immune response. This might restore the normal physiological roles of myelin proteins and should significantly slow, if not halt, the progression of the disease as well as allowing remyelination to occur. The direct use of antigenic peptides from MOG protein as therapeutics is limited by the intrinsic instability of peptides *in vivo* and their poor bio-availability. In order to stabilise these peptide sequences and engineer lead molecules for the treatment of MS, we have bioengineered the antigen sequences by grafting them onto a framework of a particularly stable class of peptides, the cyclotides.

Cyclotides are a recently discovered family of plant peptides of about 30 amino acids with a cyclic peptide backbone and six cysteine residues arranged in the cystine knot motif. These structural characteristics confer this family of peptides superb intrinsic stability through their resistance to both thermal and proteolytic degradation. The limitations of linear peptides as drugs can be overcome by using the cyclotide scaffold as a framework for novel drug design, replacing several amino acids for an active epitope. Peptide epitopes from MOG protein have been incorporated onto the cyclic framework of the model cyclotide kalata B1 by means of Boc-solid phase peptide synthesis. Nuclear magnetic resonance (NMR) chemical shift analysis was used in the first instance to assess whether the grafted analogues have folded and have a stable scaffold similar to that of kalata B1. One representative structure of a peptide with an insertion of six residues from MOG protein was determined by two-dimensional NMR spectroscopy. It shows remarkable resemblance to the native scaffold of kalata B1, which proves the incredible stability of the cylotide scaffold and its suitability as a scaffold for bioengineering.

The activity of the bioengineered peptides has been tested *in vivo*. They were tested for their immunogenicity, encephalitogenicity and protective activities by injecting them with incomplete Freund's adjuvant into transgenic mice, 24, 16 and 8 days before the encephalitogenic challenge with MOG35-55. Native kalata B1 was used as a control and each selected analogue was tested in a group of five mice. Eight weeks post-sensitisation, animals were sacrifised, blood and lymph nodes were removed for T and B cells studies (immunogenicity) and CNS tissue for histology (axonal damage, inflammation, demyelination). The course of the disease was followed by recording the clinical score from the point of the encephalitogenic challenge. For one peptide in particular, the treatment resulted in a significant decrease of the clinical score. This is an exciting result that shows the successful stabilisation of a MS epitope on the cyclic scaffold of kalata B1 and its use as a potent MS antigen. The structural information from NMR studies will be used in conjunction with activity studies in a feedback loop to design second-generation lead molecules. Development of a lead molecule for the treatment of multiple sclerosis would be a significant advance in the fight against this debilitating disease.

Daniel F Wallace

SCIENTIFIC ABSTRACT

THE LIVER IN THE REGULATION OF IRON HOMEOSTASIS: ROLE OF HEPCIDIN AND TRANSFERRIN RECEPTOR 2

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Background: The levels of iron in the body need to be tightly controlled as a deficiency can lead to anaemia and an excess can lead to tissue damage. Iron overload is usually associated with a number of genetic conditions. The most common is *HFE*-associated haemochromatosis. Recently other forms of non-*HFE* haemochromatosis have been identified caused by mutations in other genes including *hepcidin*, *hemojuvelin*, *transferrin receptor 2* (*TfR2*) and *ferroportin*. All these molecules are expressed at high levels in the liver. Recent evidence suggests that the liver is the control centre for iron homeostasis. Hepcidin is an antimicrobial peptide and iron regulatory hormone produced by the liver in response to iron overload or inflammation. Hepcidin functions by inhibiting iron release from cells and iron absorption in the intestine. Hepcidin plays an important role in haemochromatosis as its regulation has been shown to be impaired in *HFE*, *TfR2* and *hemojuvelin*-associated haemochromatosis. As well as its role in haemochromatosis hepcidin is important in inflammatory conditions such as the anaemia of chronic disease. Reliable antibodies against hepcidin have been difficult to generate and this has hindered much of the research into this area

Methods: We have produced highly specific antibodies against mouse prohepcidin. These antibodies detect prohepcidin by immunoblotting, immunofluorescence and immunohistochemistry in liver and cell lines. We have generated a mouse model of non-*HFE* haemochromatosis by knockout of the *TfR2* gene. We have analysed the phenotype and studied the expression of iron-related molecules in the *TfR2*-knockout mice. We have used the prohepcidin antibodies to study the cell biology of prohepcidin and its regulation in mouse models of haemochromatosis and anaemia including the *TfR2*-knockout mice.

Results: We have shown that prohepcidin is highly expressed in the liver and localises to the Golgi compartment and secretory pathway in hepatocytes. We have shown that *TfR2*-knockout mice develop iron overload with a similar phenotype to human *TfR2*-associated haemochromatosis. In mouse models of haemochromatosis: *Hfe*, *beta-2-microglobulin* and *TfR2*-knockout mice there is no upregulation of prohepcidin protein in the liver despite increased iron stores indicating that the regulation of prohepcidin expression is impaired in these mice. In a mouse model of iron deficiency anaemia, the sex-linked anaemia (*sla*) mouse which has a mutant hephaestin gene, prohepcidin expression is undetectable in the liver consistent with iron deficiency.

Conclusions: We have developed highly specific prohepcidin antibodies and have demonstrated that prohepcidin can be detected in the secretory pathway of hepatocytes consistent with a liver expressed secreted peptide. With these antibodies we have shown that prohepcidin protein expression is impaired in mouse models of haemochromatosis. The generation of a *TfR2*-knockout mouse has shown that in common with other forms of haemochromatosis there is a defect in the hepcidin regulatory pathway leading to inappropriately low levels of hepcidin and high levels of iron absorption in relation to iron stores.

Future directions: It is clear that the liver expressed peptide hepcidin plays a key role in the regulation of iron homeostasis. We aim to continue our studies to dissect the role of the haemochromatosis related molecules Hfe and TfR2 in the regulatory pathways involved in hepcidin induction and to study the role of hepcidin in other common and clinically important disorders such as the anaemia of chronic disease and anaemia of the elderly.

Katherine T. Andrews

Antiretroviral drugs as anti-malarials

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Malaria is a parasitic disease transmitted by mosquitoes and affecting 300-500 million people annually. The most lethal type of malaria is caused the parasite *Plasmodium falciparum* and it is estimated that 1-2 million people die each year as a result of infection with this *Plasmodium* species. Most of the deaths caused by *P. falciparum* infection occur in sub-Saharan Africa, but deaths also occur in other regions of the world including Asia and America. Unfortunately, there is currently no vaccine to protect people against malaria, and existing anti-malarials (eg chloroquine) are becoming less effective due the development of parasite resistance. New drugs that act on essential parasite processes are attractive targets for development of therapeutics to combat drug resistant parasites.

One such malaria-specific pathway is that of haemoglobin digestion, a process necessary for normal parasite growth and development within the human erythrocyte. The enzymes involved in haemoglobin digestion include cysteine and aspartic proteases. We have taken a novel approach in the investigation of malaria aspartic proteases as potential anti-malarial targets. We have focused our attention on aspartic protease inhibitors that are already in clinical use for the treatment of HIV/AIDS, an infectious disease that is co-endemic with malaria in large geographical regions of the world.

Our group found that 5 of 7 HIV protease inhibitors investigated, ritonavir, saquinavir, indinavir, atazanavir and Kaletra™, kill *P. falciparum* parasites in vitro. Importantly these drugs are effective within or below the plasma concentrations obtained in individuals taking these drugs for treatment of HIV/AIDS. Antiretroviral protease inhibitors are generally administered in combination, and it is likely that any new anti-malarial therapy would be administered in combination with existing anti-malarials. For this reason, and in order to minimise the development of drug resistance, we have examined the anti-malarial activity of various drug combinations. When saquinavir was tested together with the anti-malarial chloroquine, an additive antimalarial action was observed. In contrast ritonavir/saquinavir and ritonavir/chloroquine combinations displayed synergistic anti-malarial activity, meaning that the effect of the two drugs together was significantly better than the effect of each drug when used alone.

A necessary step in the development of drugs for any potential therapy is demonstrated efficacy in an in vivo model. To test this, we examined the anti-malarial effect of a panel of HIV protease inhibitors in mice infected with the non-lethal rodent malaria, *P. chabaudi* AS. We achieved a significant reduction in the median peak parasitemia of mice treated orally with ritonavir/saquinavir (2% compared to 20% in the vehicle control group; $P=0.01$) or ritonavir/lopinavir (4% compared to 20% in the vehicle control group; $P<0.004$). These results were obtained at drug levels well below those clinically achieved in humans taking these drug combinations for HIV/AIDS therapy. Mice given ritonavir alone also showed a significant reduction in median peak parasitemia compared to the vehicle control group (9% compared to 20% in the vehicle control group; $P=0.037$), however treatment with saquinavir did not significantly affect parasitemia, a result which was expected given that saquinavir is rapidly metabolised in vivo unless administered with a cytochrome P450 inhibitor such as ritonavir.

The observed inhibitory activity of antiretroviral protease inhibitors against malarial parasites, *P. falciparum in vitro* and *P. chabaudi in vivo*, raises the prospect of their use as anti-malarial drugs. They have the advantage over new experimental anti-malarial agents in development as they are already clinically available drugs that are widely prescribed for HIV/AIDS. In addition, due to increasing multinational agreements in recent years, highly active antiretroviral therapy is increasing in regions where HIV and *P. falciparum* are both endemic. Our findings have important implications for treatment strategies in co-endemic settings and suggest that appropriate choice of HIV drug combinations may also have an important role to play on malaria disease outcome.

Dagmar Wilhelm

Boy, girl, or a mix of both:
A molecular and cellular mechanism to reinforce testis development in the male

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Reproductive fitness and social mores are tightly geared to the existence of two distinct sexes, male and female. The arrival of an intersex baby is commonly treated as a medical emergency, and intersex is heavily stigmatised in our society. Disorders of sexual development in humans are surprisingly common, ranging from mild conditions like hypospadias (inappropriate location of the urethral opening in approx. 4% of male births) to complete sex reversal (1 in 20 000 births). The overall costs of surgical intervention and patient management contribute significantly to the national health budget. However, most of these disorders are still unexplained at the molecular level.

Our research has uncovered a molecular and cellular mechanism that operates to ensure correct development of the testes, even when the primary testis-determining switch in the male embryo is impaired. In mammals, the Y-chromosome gene *SRY* acts as the initial trigger of the cascade of gene regulation responsible for testis differentiation. The location of *SRY* on the Y chromosome makes it particularly vulnerable to mutation, since normal repair mechanisms that protect other genes present in two copies cannot operate, and yet a surprisingly low proportion of sexual disorders can be attributed to mutations in the *SRY* gene.

Despite the pivotal role of *SRY*, we know almost nothing about its molecular mode of action, largely because of the lack of molecular probes for these analyses. We have raised a novel and unique antibody specifically recognizing endogenous mouse SRY protein, and used it to investigate the molecular and cellular mode of action of SRY in testis determination. We find that SRY protein is expressed in a wave in the XY mouse fetal genital ridge (the gonadal primordium) beginning approx. 10.5 days *post coitum* (dpc), and ending approximately 12.5 dpc. The subset of somatic cells that expresses SRY begins to express the transcription factor SOX9 almost immediately, marking these cells as pre-Sertoli cells and signalling the beginning of testis differentiation.

Curiously, we found that a small proportion of SOX9-positive cells in the early XY gonad did not express SRY, prompting us to investigate the mechanism whereby *Sox9* expression might be upregulated in these cells. We confirmed by *ex-vivo* XX-XY cell mixing experiments, that XY cells are able to engage receptor-mediated signalling to up-regulate *Sox9* expression in *Sox9*-negative (XX) cells. Finally, we showed by employing specific inhibitors in mouse fetal gonadal organ culture that the causative signalling molecule is prostaglandin D_2 (PGD_2), and that PGD_2 can induce *Sox9* expression in cultured XX gonads.

Our data indicate a mechanism whereby *Sry* uses both a cell-autonomous mechanism and a PGD_2-mediated signalling mechanism to stimulate expression of *Sox9* and induce the differentiation of Sertoli cells *in vivo*. Once a proportion of cells is induced to develop as Sertoli cells by the cell-autonomous upregulation of *Sox9*, these are able to recruit other cells of the same lineage to adopt a Sertoli cell fate, preventing them from developing as ovarian granulosa cells. This interpretation is consistent with the existence of discrete testicular and ovarian regions exist in ovotestes, as opposed to a “salt-and-pepper mixture” of testicular and ovarian cell types. We suggest that this represents an important mechanism for canalization of gonad development to the testicular *vs* ovarian pathway, even when SRY activity is reduced and may explain the low frequency of Sry-related intersex disorders in the human population.