ROUNDUP360

Research

Sometimes developments in the general scientific field are so significant that here at *360* we feel they merit bringing to the wider orthopaedic community. There have been two very significant developments in the way that we understand the human genome published in recent months, so significant in fact that even the resident *360* boffins were taken slightly by surprise.

Beyond the human genome?

Traditional genetic teaching from high school onwards focuses around a genetic theory developed from Mendel's early foundation: that genes are inherited in a predictable pattern. It is commonly accepted that genes are encoded on DNA and that they are made up of a combination of control sequences (promoters), formed from coding (exon) and non-coding (intron) regions. When the RNA template is produced the introns are removed and their coded information does not go into the peptide sequence making the protein from the gene. The exons only form 1.5% of our genome, and the rest was, until relatively recently, thought to be "junk DNA", possibly from defunct genes and previous evolutionary blind alleys. The ENCODE project co-ordinated through researchers in **La Jolla (USA)** aims to turn this view on its head. The Human Genome Project looked at sequencing and the function of the 23 000 or so genes that form the exome. ENCODE is a collaborative project between 30 genomic institutes that is starting to show that as much as 60% of the genome may be involved in protein synthesis, mostly in the regulation of the exome through a much more complex arrangement of switches and other control mechanisms. The ENCODE project is helping to explain anomalies such as the statement: "Humans are genetically 98% similar to a chimpanzee", which is correct, but much of what makes us different is in the control of that exome.[1] There are many areas of orthopaedic science that are only partly explained by the genetic patterns we see, such as the propensity to develop osteoarthritis, for implants to loosen or for patients to be able to survive a massive physiological insult such as major trauma. Here at *360* we feel sure that a clearer understanding of the upstream and downstream transcription regulation of the variety the ENCODE project is providing will see a revolution in the way we understand the genetics of our orthopaedic patients. We raise a glass (or two!) to these pioneers of genetic science.

New RNA... whatever next?

Another highly significant paper that may have escaped the beady eye of our *360* readership, but we are sure will not escape their interest, has identified a completely new class of RNA, which functions like genes. Traditional understanding of the role of RNA is that it plays a part in transcription of DNA into protein, through the assembly of the template, encoding apparatus and in some of the downstream regulation. Researchers in **Boston (USA)** have identified a new function for so-called long intergenic noncoding RNA (lincRNA). These lincRNAs have been previously described, but their function is relatively unclear. The research team decided to comprehensively classify the biological function of transposable element insertions (TEs). These TEs are like a form of genetic parasite (or selfish genes) and are capable of inserting their own sequence into genomic DNA. They do potentially have some subtle regulatory effects and the research team used TEs to examine the potential function of lincRNA. They examined over 9000 human lincRNAs and established that the instance of TEs was significantly higher and that in many cases these genetic parasites had inserted functional DNA into the lincRNA non-coding regions. The research demonstrated that these lincRNA genes are particularly active in stem cells and it seems likely that part of the function of stem cells is derived from the function of their constituent lincRNAs.[2] While we are no more genetic biology experts than we are rocket scientists, here at *360*, we can easily see the potential for expanding our understanding, particularly, of developmental disease and developing stem cell and tissue biology functions. It seems to us that a clear understanding of what drives stem cell function is required if we are to be able to fully harness the potential of this emerging technology in the ways described in our two feature articles this month.

Cells, matrix and gene enhancement

There is something about the belt and braces approach with difficult-to-treat conditions or complications. We as a surgical community seem to like to try everything, or as the British would say, "everything but the kitchen sink". So we certainly raised a metaphorical eyebrow when we came across this study from researchers in **Qingdo (China)**. Having identified that osteochondral defects can be challenging to treat in young knees, the research team aimed to evaluate the efficacy of the 'kitchen sink' approach. They studied a surgical technique which included using a multimodal therapy, consisting of mosaicplasty and injection of hIGF-1 via a plasmid vector in a calcium alginate carrier medium in a goat animal model. Health cartilage harvested from the intertrochlear groove was used to address the defect and bare areas were treated with gene-modified BMSCs–scaffold complex. The study involved 56 healthy goats, each with a surgically-created 6 mm osteochondral defect. The goats were divided into three groups to test the efficacy of mosaicplasty, moasicplasty and gene therapy, and no treatment. Outcomes were assessed with MRI and microscopically at four and 16 weeks.[3] While the results of this approach appeared encouraging, the only real differences found between the mosaicplasty and mosaicplasty plus gene therapy groups, were in a slightly better gross appearance; all quantitative

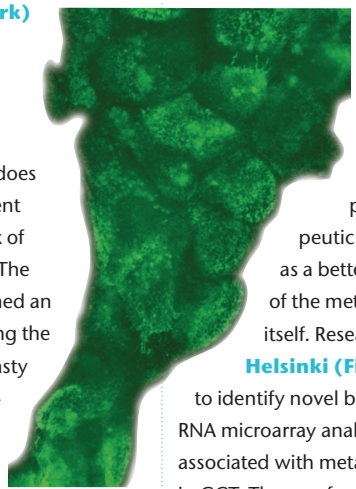
and qualitative measures showed no apparent difference in the quality of the cartilage formed. Gene therapy, growth factors and other biologic agents certainly show promise, but for us at *360* this study illustrates the current state of knowledge brilliantly; biologic agents certainly do no harm, but it is unclear whether the healing process is the surgery or the biology.

Histology of x-rays: cement bone interfaces revisited

Arthroplasty surgeons are beloved of their radiographs, looking for any signs of the dreaded radiolucent line, certain that this may herald impending doom for the patient. Those knee surgeons who have been performing the Oxford Unicompartmental Knee replacements have, however, had to hold faith in the presence of rapidly developing radiolucent lines that have divided opinion on the success or otherwise of the unicompartmental knee. Do radiolucent lines mean loosening with this prosthesis? A definitive answer to this question would settle a lot of nerves in arthroplasty clinics the world over. A research team from **Oxford (UK)** set out to establish what exactly was going on at cellular level in the bone–cement interface. The study team retrieved specimens from the removed tibial components of ten patients undergoing revision of their unicompartmental knee replacement with a well fixed tibial tray which was removed *en bloc*, together with the underlying bone to allow analysis of the interface. All of the patients had radiolucent lines under their tibial tray, varying from partial to complete lines. The researchers, however, found that all trays had areas of bone/cement contact ranging from 19% to 95%. The team also noted that the extent of the radiolucent line was inversely proportional to the bone–cement contact area.[4] Although the findings of this study should be eyed with a little suspicion, the designing centre does have a significant vested interest in proving the worth of their prosthesis; after all, we are reassured here at *360* that the presence of a radiolucent line does not have the same ramifications for the patient or surgeon that it does in total hip replacement.

THR and VTE in the Danish population

The controversial, commercial and clinical debate that surrounds the use of antithrombotic agents in patients undergoing surgery in general, and in particular total joint replacement surgery, continues to occupy much of the world's medical press. One of the difficulties that we have had here at *360* in balancing the risk of complications (particularly with extended thromboprophylaxis) and risk of the sequelae of venous thromboembolism (SVTE), is that there is little comparative data. The rate of thromboembolic disease in the background population is simply not known. Researchers in **Aarhus (Denmark)** have been beavering away with their population registries to answer the simple question: how long does a total hip replacement put you at excess risk of thromboembolism? The research team designed an impressive study using the Danish Hip Arthroplasty Registry. They identified 85 965 patients who underwent THR over a 15-year period, and recorded their demographics, venous thromboembolism data and the prophylaxis the patients received. They used a comparison cohort from the Danish Civil Registration system of patients who had not had a THR. The patients within their THR cohort had inpatient (but not extended) thromboprophylaxis 97% of the time. The researchers found a significant increased risk of SVTE, both within 90 days of the surgery (0.79% *versus* 0.05%) and for the rest of that year (0.29% *versus* 0.12%). This equates to an adjusted relative risk of 15.84 at 0 to 90 days and 2.41 at 91 to 365 days. The research team were unable to find any effect of potential risk factors including age, comorbidities and gender.[5] The information contained in this study is superb. Although the relative risk is high pre-90 days, the absolute risks are small, and certainly beyond 90 days the absolute excess risk of an SVTE event was only 0.17%. We wonder what the excess complications and decline in SVTE rates would be with use of extended thromboprophylaxis?



Potential therapeutic targets for GCT

Giant cell tumour (GCT) is an unusual beast. Mostly locally aggressive but relatively easy to treat, the tumour can turn nasty and classically metastasises to the lungs. Metastatic GCT is a completely different tumour and understanding the process by which the tumour differentiates to metastasise may give new insights into potential therapeutic targets, as well as a better understanding of the metastatic process itself. Researchers from **Helsinki (Finland)** aimed to identify novel biomarkers using RNA microarray analysis that were associated with metastatic change in GCT. They performed an *ex vivo* human tissue study using tissue from ten primary cases of GCT, five of which went on to develop metastatic lung deposits. The researchers used an RNA expression analysis technique based around the taqMan process to measure expression of miRNAs. The researchers identified 12 miRNAs that were differentially expressed in the metastatic group, and then confirmed these findings with qRT-PCR. The researchers found eight genes that were inversely expressed in relation to their miRNA regulators. The NFIB gene was of particular interest as NFIB (a nuclear binding protein involved in DNA replication) expression, and further analysis was undertaken with immunohistochemistry on an archive cohort of 74 cases of GCT.[6] The group confirmed their initial suspicions that NFIB and its regulator miR-136 are suitable for use as prognostic markers for metastatic spread, and given time, perhaps novel targets for chemotherapy.

Optimising vancomycin elution from cement

Getting the best outcome for a patient following an infection can be tricky. Cement has a big role to play in a variety of surgical options from total joint replacement to the Masquelet technique, and as temporary spacers. Improving antibiotic elution characteristics is a bit of a balancing act: too much antibiotic and the mechanical properties of the cement will be degraded, compromising the patient's outcome by increasing the chances of cement fracture and failure; too little antibiotic and the infection will be less effectively combated. We were delighted to read a very simply conceived, but carefully executed, study from **Kansas City (USA)** with a very clear clinical message. The research team aimed to assess the best method for ensuring antibiotic elution without degrading the mechanical properties of cement. They chose to look at things a little differently from previous work. Instead of varying the quantities and type of antibiotic, they decided instead to vary the volume of monomer and the timing of the antibiotic addition as well. They used a well established protocol for testing the biomechanical and antibiotic elution characteristics of the cement bars they produced. They used Simplex P and SmartSet MV bone cement and undertook three different mixing protocols; standard technique, double monomer, and delayed antibiotic addition. They established that delaying the addition of the antibiotics by 30 seconds increased the elution significantly over a six-week period with no significant difference in strength,

while increasing the monomer reduced the elution characteristics. The delayed antibiotic administration group increased elution by 52% in Simplex and 25% in SmartSet MV.[7] We at *360* applaud the authors for a fantastically simple study that has an immediate application to clinical practice. We are already delaying our antibiotic addition and hope you will too.

How much sleep is enough?

Throughout the world the situatution for surgeons in general, and trainees particularly, is improving with regards to working practices. But amazingly it's the surgeons themselves who have been most resistant to this change. How many other professions, or specialties within medicine for that matter, would complain that they want to work harder, and take less time off? We are all, it seems, gluttons for punishment. So why are we being forced, throughout the world from the US to Australia to the UK, to regulate our working patterns? Researchers in **Baltimore (USA)** set up a study to establish the effects of sleep deprivation on surgeons' cognitive and psychomotor abilities. The research team performed a prospective study of all grades of surgeon using validated psychomotor and cognitive functional tests. The tests were conducted over two four-week periods and a multivariate analysis was used to establish the impact of covariates on outcomes including sleep obtained the night before.[8] The research team established that surgeons receiving less than four hours sleep had a 1.43 increased chance of making a mistake across all testing. When specifically assessing attention and concentration, and adjusting for confounders, there was a 72% increase in the chance of making an error after less than four hours sleep. There were no significant differences in other tests. It is unclear to us here at *360* how this relates to decision making and surgical aptitude, although the data would suggest we should strive to ensure our trainees receive a minimum of four hours sleep if any critical task is to be performed the next day.

REFERENCES

1. **Ecker JR, Bickmore WA, Barroso I, et al.** Genomics: ENCODE explained. *Nature* 2012;489:52-55.
2. **Kelley DR, Rinn JL.** Transposable elements reveal a stem cell specific class of long noncoding RNAs. *Genome Biol* 2012;13:R107.
3. **Leng P, Ding CR, Zhang HN, Wang YZ.** Reconstruct large osteochondral defects of the knee with hIGF-1 gene enhanced Mosaicplasty. *Knee* 2012;19:804-811.
4. **Kendrick BJ, James AR, Pandit H, et al.** Histology of the bone-cement interface in retrieved Oxford unicompartmental knee replacements. *Knee* 2012;19:918-922.
5. **Pedersen AB, Johnsen SP, Sørensen HT.** Increased one-year risk of symptomatic venous thromboembolism following total hip replacement: a nationwide cohort study. *J Bone Joint Surg [Br]* 2012;94-B:1598-1603.
6. **Mosakhani N, Pazzaglia L, Benassi MS, et al.** MicroRNA expression profiles in metastatic and non-metastatic giant cell tumor of bone. *Histol Histopathol* 2012;(Epub ahead of print) PMID: 23172052.
7. **Amin TJ, Lamping JW, Hendricks KJ, McIff TE.** Increasing the elution of vancomycin from high-dose antibiotic-loaded bone cement: a novel preparation technique. *J Bone Joint Surg [Am]* 2012;(Epub ahead of print) PMID: 23014891.
8. **O'Brien MJ, O'Toole RV, Newell MZ, et al.** Does sleep deprivation impair orthopaedic surgeons' cognitive and psychomotor performance? *J Bone Joint Surg [Am]* 2012;(Epub ahead of print) PMID: 23014765.