HIV from the prostate, testis, semen and blood to more accurately pinpoint where this second compartment of infection lies. 'The impact on the direction of research on AIDS vaccines and treatments could be significant', concludes Gupta.

REFERENCES

1 Collins, K.B. *et al.* (2000) Development of an *in vitro* organ culture model to study transmission of HIV-1 in the female genital tract. *Nat. Med.* 6, 475–479

2 Bomsel, M. (1997) Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. *Nat. Med.* 3, 42–47

3 Gupta, P. *et al.* HIV-1 shedding pattern in semen correlates with the compartmentalisation of viral quasispecies between blood and semen. *J. Infect. Dis.* (in press)

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Mouse model of spinal muscular atrophy

Researchers from Ohio State University (OSU; Columbus, OH, USA) have for the first time replicated the condition, spinal muscular atrophy (SMA), in a mouse model and found that large quantities of the protein Survival Motor Neuron 2 (SMN2) corrects the SMA phenotype[1]. Their findings support the conclusion that SMN2 could act to prevent the damage caused by SMA, which has led to the search to isolate a compound that will activate production of SMN2 to treat SMA.

Spinal muscular atrophy (SMA) is the most common inherited cause of childhood mortality, with an incidence of 1 in 10,000 live births[2]. It is an autosomal recessive disorder, with one in every 40 people carrying the gene that causes SMA (Ref. 3), and it is characterized by the destruction of the α-motor neurons in the spinal cord that control voluntary muscle movement.

The genetic basis of SMA

SMA is caused by mutations in the telomeric survival motor neuron gene *(SMN1)*, but patients retain at least one copy of a highly homologous gene, centrometric SMN (*SMN2*; Fig. 1)[4]. Both genes are found on chromosome 5 where they are located ≈500 kb from each other. 'Sequence analysis of the SMN genes show there is only one functional nucleotide difference between the two genes. This lies in exon 7 and affects splicing,' explains Arthur Burghes, Associate Professor in the Departments of Neurology, Medical Biochemistry and Molecular Genetics, OSU, and the lead investigator of the project. The result is that for the *SMN1* gene, approximately 90% of the transcript is full length containing exon 7, but for the *SMN2* gene, only 10% of the transcript contains exon 7.

The significance of exon 7 is that it contains crucially important information for the SMN protein. One function that is affected by the deletion of this exon is the ability of the SMN protein to oligomerize. This single difference causes the quantity of protein produced to differ between the two loci. The result is that the *SMN1* gene produces high levels of SMN protein, while the *SMN2* gene does not produce sufficient SMN protein (Fig. 2). Increasing the number of *SMN2* genes present, however, results in the production of more SMN protein[1].

Humans carry different numbers of the *SMN2* gene as unequal crossing over at meiosis results in some people carrying two copies of the *SMN2* gene and others having none, while most have one copy. 'If you are carrying an extra copy of the gene, you will double the quantity of SMN2 protein produced,' says Arthur Burghes. This results in a reduction in the severity of the SMA symptoms.

Species other than humans have only one SMN gene that is the equivalent of the human *SMN1* gene. In mice, a homozygous knockout of the SMN gene results in early embryonic lethality following massive cell death. Coupled with the fact that SMA patients lacking the *SMN2* gene have never been reported, this suggests that SMN plays an essential role during embryonic development.

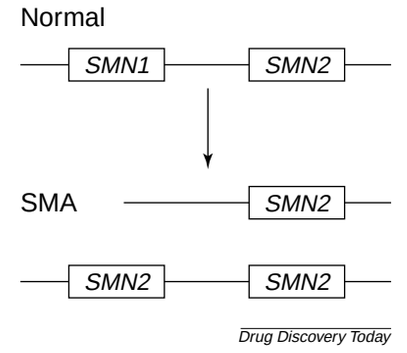

Figure 1. *Diagram of mutations giving rise to spinal muscular atrophy (SMA). The loss of the SMN1 gene can occur by deletion or conversion of SMN1 to SMN2.*

Mouse model of SMA

In collaboration with Michael Sendtner's group from the University of Würzburg (Würzburg, Germany), Burghes and his team have created a mouse model by introducing the entire human *SMN2* gene onto a null SMN background. This mimics the situation in human SMA patients, where *SMN1* is

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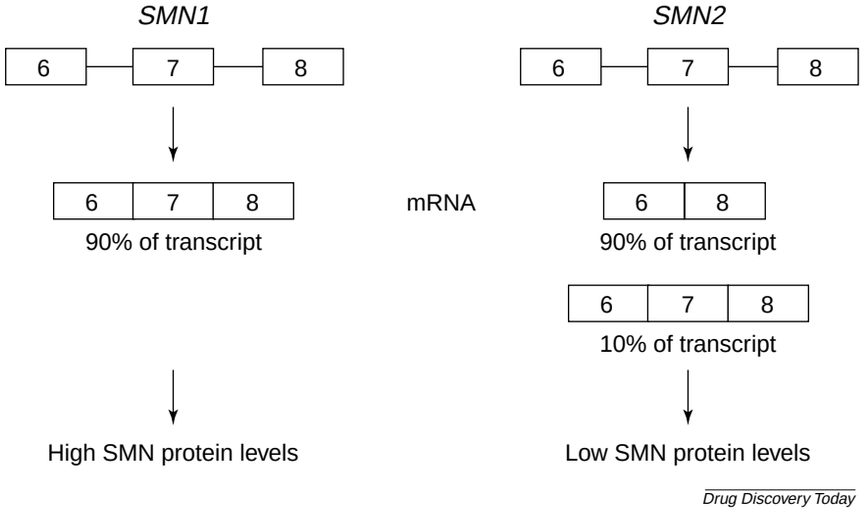

Figure 2. *The difference between the SMN1 and SMN2 gene results in SMN2 producing insufficient quantities of SMN. Increasing the copy number of SMN2 results in an increase in the total quantity of full-length transcript (10% of the SMN2 copy number) and therefore an increase in the quantity of SMN protein which, when it reaches sufficient levels, can correct the SMA phenotype.*

absent but *SMN2* is present and low SMN protein levels are observed.

The team showed that mice carrying 1–2 copies of the transgene have normal numbers of motor neurons at birth, but vastly reduced numbers by postnatal day 5, and subsequently die. This closely resembles the severe type I SMA phenotype in humans and is the first report of an animal model of the disease. Eight copies of the transgene, however, rescues this phenotype in the mice, indicating that phenotypic severity can be modulated by *SMN2* copy number.

'Here for the first time we have been able to show that there is no loss of motor neurons at birth, but that the problems develop around day three in mice. So theoretically, if we could find ways to activate the *SMN2* gene early in life, we might prevent the loss of motor neurons,' explains Burghes.

Future projects

Delivering the SMN protein would not be practical, as it does not cross the blood–brain barrier, and it would be hard to ensure that it gets to the area where it exerts its action. Instead, the team are undertaking high-throughput screens for compounds that might activate the *SMN2* gene. There are three possible ways in which this might work. Drug compounds could act as transcriptional activators increasing the quantity of SMN message produced and therefore the quantity of SMN protein. The second possibility is that it might be possible to inhibit the negative element in the SMN promoter to increase expression. Thirdly, splicing enhancers could be used to stimulate the inclusion of exon 7 into the transcript.

The team is currently running high-throughput screens of 40,000 drugs against cells from a transformed fibroblast line from an SMA patient to search for compounds that can increase the number of particles of SMN, known as gems. Families of SMA patients have recently signed a letter indicating their intent to collaborate with Aurora Biosciences (San Diego, CA, USA), who will be developing ultra-HTS assays to rapidly screen hundreds of thousands of potential compounds for their ability to activate SMN2 production.

REFERENCES

1 Monani, U.R. *et al.* (2000) The human centromeric Survival Motor Neuron gene (*SMN2*) rescues embryonic lethality in *Smn*$^{-/-}$ mice and results in a mouse with spinal muscular atrophy. *Hum. Mol. Genet.* 9, 333–339

2 Pearn, J. (1973) The gene frequency of acute Werdnig-Hoffman disease (SMA type 1). A total population survey in North-East England. *J. Med. Genet.* 10, 260–265

3 Pearn, J. (1978) Incidence, prevalence, and gene frequency studies of chronic SMA childhood spinal muscular atrophies. *J. Med. Genet.* 15, 409–413

4 Lefebvre, S. *et al.* (1995) Identification and characterization of a spinal muscular atrophy-determining gene *Cell* 80, 155–165

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