are sulfated and that these moieties are important in HIV-1 entry. 'We wanted to understand the role of sulfation and how crucial it is to binding of the envelope glycoprotein to CCR5', says Paul Maddon (Chairman and CEO, Progenics). The collaborative team therefore obtained synthesized sulfated peptides, and found that substitution of the sulphate groups with phosphate groups (also negatively charged at physiological pH) abolished inhibition of gp120/CCR5 binding, thereby demonstrating the importance of the sulfation and showing that this was not an electrostatic effect. Furthermore, inhibition of gp120/CD4 binding was dependent on the correct primary structure surrounding the sulfotyrosines. 'This region of CCR5 is a specificity determinant', concludes Dragic. 'The other parts of CCR5 are not required for gp120 docking, but they might be needed to get a tighter binding.'

Dimiter Dimitrov, Senior Investigator at the NCI-Frederick Cancer Research and Development Center, National Institutes for Health (Frederick, MD, USA) was positive about the findings saying, 'The quality of the research is outstanding.' He added, 'Previous attempts by several groups including my own to use N-terminal peptides as inhibitors failed because they were not sulfated. There is already a precedent for a peptide that has been quite successful in clinical trials as an inhibitor of HIV-1 entry – T20. Although the sulfated N-terminal CCR5 peptides are not as potent inhibitors as T20, they could provide a basis for the development of more potent inhibitory peptides or small molecules.'

The next project will continue the search for the exact specificity determinants using mutant or modified peptides, to reduce the peptide length and increase the affinity.

HIV-1 entry

The effect of these tyrosine modifications on HIV-1 entry was also examined through the use of luciferase-expressing reporter viruses. The results showed a partial (≈50%) inhibition of viral entry by sulfotyrosine peptides, this low level possibly explained by the inaccessibility of the CCR5-binding sites on gp120 (Ref. 1). The workers suggest that after the gp120/CD4 complex is formed and the CCR5-binding site is exposed or created, gp120 might preferentially interact with the membrane-bound coreceptor rather than the soluble CCR5 nucleotide peptide.

Future studies

Progenics are now using these peptide structures to screen for drugs that inhibit gp120 binding to this region of CCR5. In the longer term, the company plans to optimize the peptide structures to increase their efficacy in inhibiting HIV-1 entry. 'We hope that this project will produce some lead compounds for novel HIV-1 therapies in 2001,' anticipates Maddon. The next stage for Dragic's group is to try to mimic this research with the other main HIV-1 entry co-receptor, CXCR4. However, Dragic suggests that, 'This might be more difficult, as the binding site is more diffuse, covering residues from both the extracellular loops and the amino terminal domain.'

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Signalling in Huntington's disease: novel targets identified

A collaborative study conducted by the Fred Hutchinson Cancer Research Centre (Seattle, WA, USA) and the Massachusetts General Hospital (Boston, MA, USA) has identified changes in nerve cells in the early stages of Huntington's disease (HD) using a mouse model. Using microarray technology, the team have identified several signalling molecules that could provide future targets for therapies[1].

HD is an autosomal dominant neurodegenerative disorder with mid-life onset that is characterized by psychiatric, cognitive and motor symptoms. Chorea (meaning 'mad dance'), is the most common involuntary movement in adult HD patients, with deficits in attention and memory often present at the time of onset of motor dysfunction. The prevalence of HD in Northern America and Europe is 5–10 per 100 000 of the population. Currently, there is no cure for HD and no therapeutic approach to delay the onset of symptoms, and death can occur within 12–15 years.

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Mechanisms underlying HD

The HD mutation was identified in 1993 as an unstable expansion of the trinucleotide, CAG repeats within the coding region of the gene *IT15* (for Interesting Transcript), and produces an expanded stretch of glutamine residues attached to the amino-terminal. Individuals with 35 or fewer CAG repeats on the longest allele will not develop HD, those with 36–39 might develop HD, while those with 40 or more will almost certainly develop the disease. The number of repeats correlates inversely with age of onset or with age at death.

The function of the normal huntingtin protein and the mechanism of pathogenesis caused by the polyglutamine expansion are unknown. It is, however, known that huntingtin is required for normal development as mouse knockouts of the gene are embryonic lethal[2].

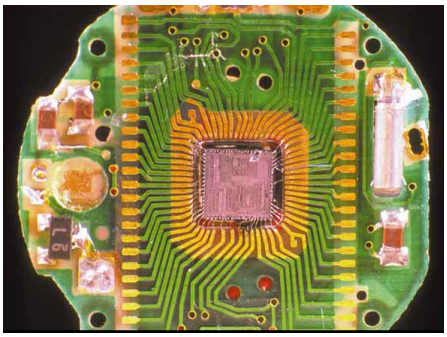
Although the abnormal huntingtin is ubiquitous throughout the body, the pathology of HD is restricted to the brain where degeneration occurs initially in the striatum and cortex. The medium spiny GABAergic cells of the striatum appear to be preferentially damaged in HD.

Positron emission tomography (PET) studies of presymptomatic human patients indicate that neurotransmitter receptor levels decrease prior to the onset of overt clinical symptoms[3]. Similar decreases have been shown in a transgenic mouse HD model where the receptors are selective (i.e. only certain receptors are decreased)[4]. The pattern of neurotransmitter receptor decreases appears to reflect downregulation of a specific subset of genes. The receptor changes, which occur at the levels of both protein and mRNA, precede and therefore could contribute to the onset of clinical symptoms.

'After animal models of HD were created, we realised that signs of cellular damage occur prior to neuronal loss. We decided to look at cells upstream of cell death to see what the scope of these changes might be and whether it might be possible to reverse such processes pharmacotherapeutically,' says Ruth Luthi-Carter from the Department of Neurology at the Massachusetts General Hospital and first author of the study.

Microarray studies

The team licensed the Affymetrix (Santa Clara, CA, USA) microarray technology (GeneChips®) to profile 6000 striatal mRNAs in the R6/2 mouse model (pro-



duced by introducing exon 1 of the human gene with an expanded CAG repeat into the mouse) of HD. The mice were studied at both 6 and 12 weeks of age, representing stages of minimal and pronounced deterioration in neurological function and compared with their wild-type littermates. Researchers dissected out material from the striatum, pooled material from six mice to produce a large enough quantity, and extracted RNA using phenol.

They exposed these samples to the microarrays and labelled the RNAs with a fluorescent dye. The location and intensity of light emitted by the dye was measured using a computer-controlled laser and the results indicate which genes are active and the extent of their activity.

The researchers found that of the 144 genes whose expression was different between the wild-type and transgenic animals, 72% showed decreases in HD animals, and 28% showed increases. 'Some of these alterations in gene expression will undoubtedly be "red herrings". However, 38 of these changes were in the signal transduction category which suggests that something significant may be happening here,' says Luthi-Carter. In a second HD mouse model (N171-82Q), the team observed some common changes in gene expression suggesting that some are caused by the mutant Huntington's gene.

The next step

In total, there are approximately 12 different mouse models of HD that a consortium of 50 scientists (the Huntington's Disease Array group) now plan to study using these microarrays. 'If the various models show common abnormalities in gene expression, we will then try to find pharmacotherapeutic agents that will counteract the deficiency or excess of these particular molecules,' says Luthi-Carter.

In addition, the team plans to examine human samples using the microarrays to see whether any of the changes in gene expression correspond with mouse models. However, it could be difficult to identify suitable human samples, as the affected neurons from those that have died of HD, have already succumbed to the disease making it impossible to reconstruct the pathological process that killed them. Hence, the team hopes to use autopsy material from individuals who carried the HD mutation but died from a cause other than HD.

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