A surprising recent finding demonstrated that the export process shows a high degree of specificity. 'When we experimented with glioblastoma cells in culture – cells that normally export FGF-2 – and we put FGF-1 into them, we found that the cells could not release the FGF-1,' reports Baird. Further investigations led to the discovery of the part of the sequence of FGF-2 that might facilitate its export from the cell. Baird and colleagues systematically combined different portions of the protein sequences from FGF-2 and FGF-1 and, by tracking the transport of each FGF-2–FGF-1 chimera through U87-MG glioblastoma cells, identified a specific export signalling sequence within FGF-2.

Baird speculates that cells might have specific export channels for different proteins. This suggests that different export channels might be selectively blocked, opening the possibility of different therapeutic strategies. 'We are now concentrating on a family of molecules – exhibins – that block the IL-1 export process and are investigating them for specific blocking activity using cell-based assays,' says Baird. Chemical libraries are being used to screen analogues within the exhibin family to identify potential drug candidates that work in culture. 'This is a drug discovery/validation programme at the moment,' stresses Baird. The exhibins show promise *in vitro* and are now being tested *in vivo*. One compound (CBX10913) is orally active in mice. 'At the same time, we are also working with other groups to define the molecular pathway involved in export,' adds Baird.

Future therapeutic potential

Drugs that could block protein export would have several important therapeutic uses, but work is progressing particularly well in the areas of inflammatory diseases and cancer. Exported proteins such as IL-1 and IL-18, FGF-1, FGF-2 and MIF play a central role in inflammatory diseases where they are involved in angiogenic, allergic, autoimmune and inflammatory responses that promote cell growth and reactivity. The drug candidates, CBX10913, CBX18469 and CBX21404, which show activity in preclinical models of inflammatory disease, could have potential in rheumatoid arthritis. Exhibins that inhibit the release of the exported proteins from cells are also being investigated for their potential to control autocrine (glioblastoma, melanoma) and paracrine (prostate and breast cancers) cell growth. Compounds CBX11030 and CBX10913 are currently under further evaluation in preclinical cancer models.

'Also of great interest, is the possibility of using exhibins against infectious disease,' says Baird. Gram-negative bacteria have their own protein 'export' pathway – type III secretion – that is responsible for the release of toxins. By interrupting the release of these virulence factors, it might be possible to also develop specific exhibins as novel antimicrobial agents.

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A new target for HIV-1 entry inhibition?

Despite the major impact of protease and reverse transcriptase inhibitors on HIV-1 treatment in recent years, these drugs cannot eradicate HIV-1 from infected humans. New research has described structural information on the HIV binding site that might provide a new target for the development of HIV-1 entry inhibitors. A collaboration between the Albert Einstein College of Medicine (Bronx, NY, USA), Rockefeller University (New York, NY, USA) and Progenics Pharmaceuticals (Tarrytown, NY, USA) demonstrated that HIV binds to a peptide corresponding to the

amino terminal domain of CCR5 and that this binding is dependent on the presence of sulfotyrosines at certain locations within the peptide[1].

Binding of the HIV-1 envelope glycoprotein gp120 with CD4 exposes or creates a coreceptor binding site that usually interacts with either CCR5 or CXCR4, mediating the entry of R5 and X4 HIV isolates, respectively[2,3]. Furthermore, amino acids 2–18 in CCR5 protein contain all the residues important for viral entry[4]. However, after synthesizing the peptides corresponding to these amino acids, the team, led by Tanya Dragic (Albert Einstein College of Medicine), found that they did not bind to gp120.

Sulfation of the tyrosine residues

Recent work has shown that the tyrosine residues in the CCR5 nucleotide

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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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Rebecca N. Lawrence

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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