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Gut

Leading article

Trefoil peptides and the gut

A new group of peptides has recently come to the attention of gastroenterologists: the trefoil peptides are a highly conserved group of molecules that, in the human at least, are widely distributed in gastrointestinal tissues and their primary role in man is highly likely to be in the gastrointestinal tract.

The group takes its title from the characteristic trefoil motif, a three loop structure secured by disulphide bonds based on cysteine residues (Fig 1). The supersecondary structure of the trefoil motif has been examined by 2D nuclear magnetic resonance (NMR),[1] confirming its presence and showing that it consists of a seven residue length of α helix followed by a short antiparallel ß sheet formed from two strands of four amino acids each. This is a novel supersecondary structure, clearly identifying the trefoil motif as a new class of module, distinct from other types of highly disulphide cross linked domains, such as those found in epidermal growth factor and insulin like growth factor-1.

The first molecule characterised was pS2, a secreted peptide consisting of a 60 amino acid peptide with a 24 amino acid signal peptide. It was originally discovered by differentially screening a cDNA library from MCF-7, a human breast cancer cell line.[2,3] Indeed, some 50% of all breast cancers express pS2, and this seems highly correlated with the presence of functional oestrogen receptors.[4] Far from being a breast specific peptide, however, pS2 is expressed at very low abundance in the normal breast[5]: in normal human tissues, pS2 gene expression is most prominent in the surface and foveolar cells of the stomach.[6,7]

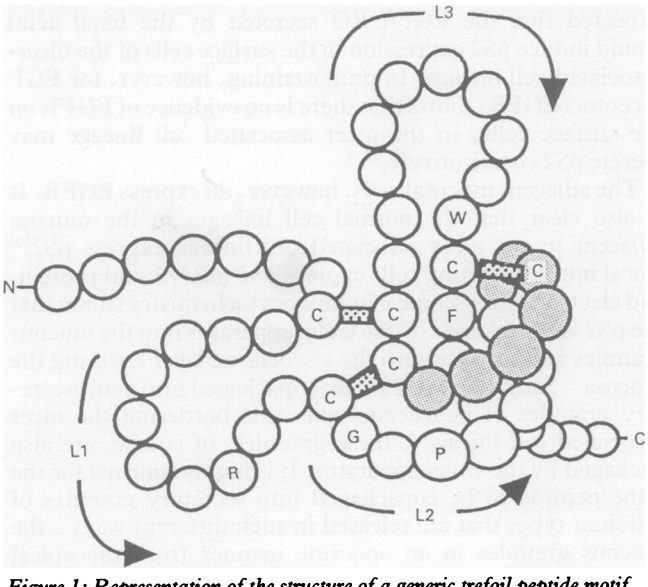

Figure 1: Representation of the structure of a generic trefoil peptide motif. The three loops are marked L1–3 and well conserved residues are shown by their single letter codes. The seven shaded residues are thought to conform to an alpha helix, and the residues at the base of L3 to be an anti-parallel β sheet.

The pS2 gene is found on the long arm of chromosome 21 (q22·3) and has three exons. Examination of the 5′ upstream sequences shows an oestrogen responsive promoter, but at −443 to −332 bases is a complex enhancer sequence that is responsive to TPA, oestrogen, the activated *jun* and *fos* oncogenes, and perhaps more importantly in the gut, to epidermal growth factor, urogastrone (EGF/URO).[8]

pS2 is found in normal gastric juice at a concentration of 30 μg/l, secreted by the surface and foveolar cells.[6] Despite this, pS2 is currently of unknown function. It is, however, highly homologous with pancreatic spasmolytic polypeptide (PSP), a larger molecule of 108 amino acids, which has two cysteine rich trefoil domains.[9] This peptide was originally found in porcine pancreatic powder,[10] and has defined physiological effects on the gut, inhibiting gastric acid secretion and intestinal motility in the rat.[11] There are high affinity binding sites in the rat intestine,[12] and binding results in inhibition of adenylate cyclase.[13] There is also evidence that PSP is a growth factor,[14] stimulating growth of colorectal carcinoma cells and MCF-7 cells in vitro.

For some time, there was no evidence for a human homologue of PSP, but Chambon and colleagues did cross hybridisation studies, starting with a 44 mer oligonucleotide based on the PSP mRNA sequence, and were able to isolate clones for mouse SP (mSP) and human SP (hSP).[15] Although pS2 and hSP are homologues, they are encoded by different genes. A functional role of hSP in humans has yet to be established, but hSP is co-expressed with pS2 in gastric foveolar cells[6] and is also expressed abundantly by the basal antral glands.[16] Moreover, in the rat, rSP is expressed by the neck cells in both fundus and antrum, and after the formation of experimental antral ulcers, rSP is induced in the basal antral glands also.[16] Thus evidence is accumulating that SP is an important antral peptide.

It is now clear that pS2 and hSP are widely expressed in gastrointestinal tissues in disease states, particularly in chronic ulcerative conditions such as Crohn's disease. Recent studies have shown that what used to be regarded as pyloric metaplasia in chronic gastrointestinal ulceration, is in fact a differentiating cell lineage that buds initially from the bases of intestinal crypts adjacent to the ulcer, and whose tubules ramify in the lamina propria before emerging from the

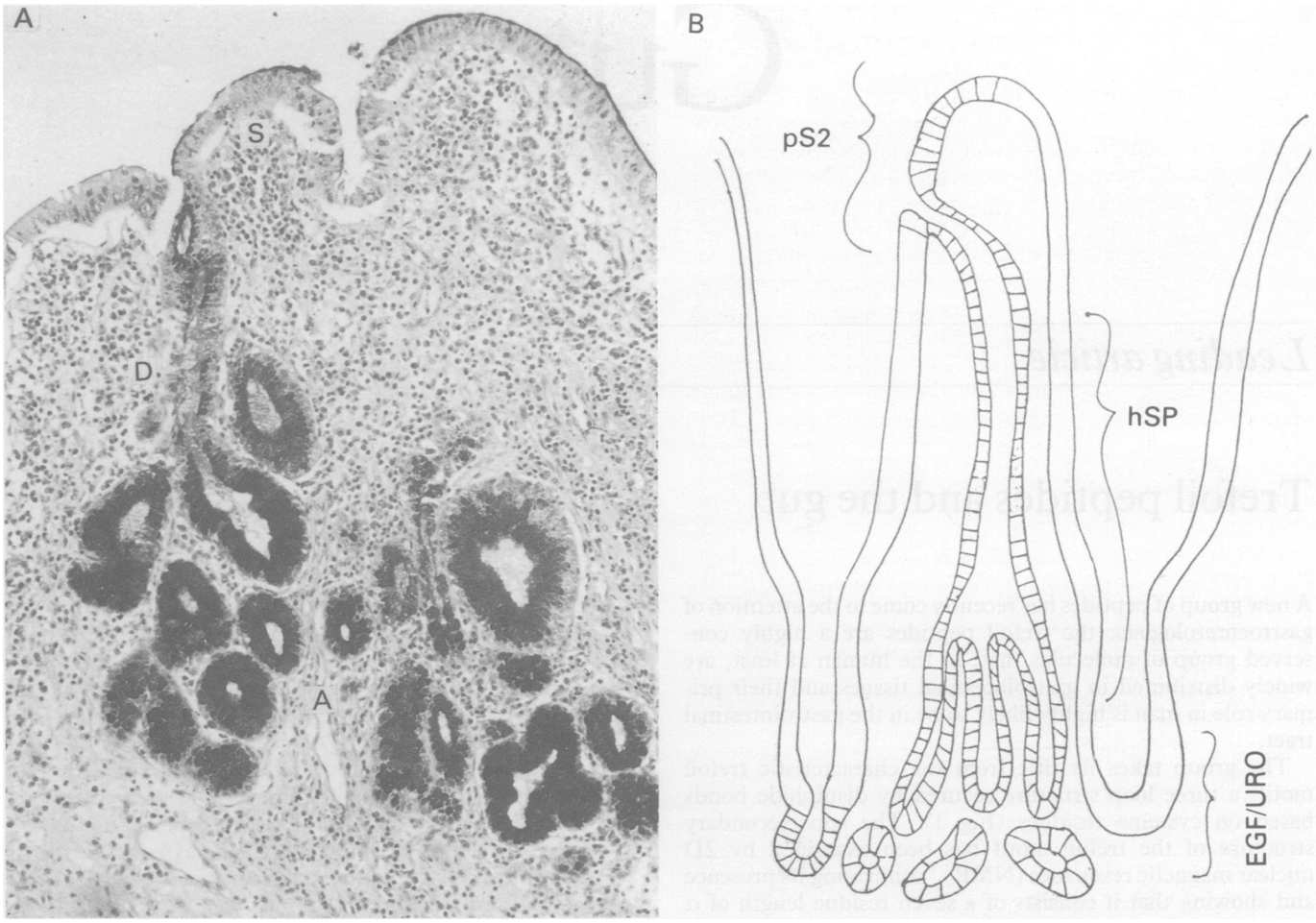

Figure 2: (A) Photomicrograph of the ulcer associated cell lineage from a case of Crohn's disease showing the acini (A), the duct (D), and the surface cells (S) (original×200). (B) Diagrammatic representation of the ulcer associated cell lineage with the secretory products of the several anatomical zones indicated (EGF/URO, epidermal growth factor/urogastrone; hSP, human spasmolytic polypeptide).

mucosal surface through a newly formed duct, which in the small intestine grows upwards through the core of an adjacent villus to emerge through a pore on the side of the villus (Fig 2).[17] The newly formed gland thus passes its secretion to the surface, and interestingly, cells from the lineage pass out through the pore to displace the indigenous cell lineages and clothe the villus surface (Fig 2). As the cells migrate through the tubular system, they acquire differentiation antigens and also develop a proliferative zone within the duct itself.[18] The glandular portion of this ulcer associated cell lineage secretes immunoreactive EGF/URO, which is then available to combine with its receptors[19] and to stimulate mucosal healing.[17]

The ulcer associated cell lineage expresses both the pS2 and the hSP genes in a site specific manner: combined immunocytochemistry and in situ hybridisation studies with ^{35}S labelled riboprobes have shown that the surface cells and the upper duct cells of the ulcer associated cell lineage express abundant pS2 mRNA and peptide, whereas hSP mRNA and protein are found in the lower duct and glandular area.[7] Thus the ulcer associated cell lineage secretes pS2 and hSP, together with EGF/URO, into the local microenvironment around the ulcer, indicating that the trefoil peptides are of considerable potential importance in mucosal healing and cytoprotection. Secretion of pS2 in Crohn's disease has already been detected through raised serum concentrations in active disease.[20]

Thus as the cells migrate through the ulcer associated cell lineage, they also sequentially change the pattern of peptide gene expression: EGF/URO in basal acini, hSP in the acini and ducts, and pS2 in the upper duct and surface cells (Fig 2). Far from being confined to the luminal gastrointestinal tract, the ulcer associated cell lineage, with its component peptides, has been reported in the pancreatic ducts in chronic pancreatitis, in the gall bladder in chronic cholecystitis, in the fallopian tube in chronic salpingitis, and also in chronic inflammatory nasal polyps.[7]

Because the pS2 gene is induced by EGF/URO, it might be expected that the EGF/URO secreted by the basal acini would induce pS2 expression in the surface cells of the ulcer-associated cell lineage. Immunostaining, however, for EGF receptor (EGFR) shows that there is no evidence of EGFR on the surface cells, so the ulcer associated cell lineage may secrete pS2 constitutively.[21]

The adjacent mucosal cells, however, do express EGFR. It is also clear that the normal cell lineages in the mucosa adjacent to the ulcer associated cell lineage express pS2.[21] Local mucin secreting cells express pS2 mRNA and protein, and electron microscopical immunocytochemistry shows that the pS2 is copackaged by the Golgi apparatus into the mucous granules and secreted into the viscoelastic layer covering the mucosa.[21] Strikingly, pS2 is also copackaged into neurosecretory granules of neuroendocrine cells bordering the ulcer associated cell lineage[21]; these granules, of course, are also packaged by the Golgi apparatus. It is highly unusual for the same peptide to be copackaged into secretory granules of different types that are released in such different ways – the mucous granules in an apocrine manner from the apical surface, and the neuroendocrine granules through the basolateral surface – where the contained peptides can act in a paracrine way on other, local cells. If indeed the EGF/URO secreted by the ulcer associated cell lineage is responsible for

the expression of pS2 in these two lineages – mucous and neuroendocrine – the reason why enterocytes do not express pS2, but do bear EGFR, is not explained.

In the colon, different trefoil peptide secreting cell lineages are also found. The cells of the hyperplastic or metaplastic polyp, long regarded as some form of hypermature colonocyte, have been shown to express both pS2 and hSP transcripts and proteins,[22] curiously in the same morphological sequence as the ulcer associated cell lineage. Whether this indicates a histogenetic linkage between the two lines is as yet unclear, but as these hyperplastic polyps increase in number with age, trefoil peptides may be secreted in increasing concentration into the colon with advancing time.

Thus trefoil peptides are secreted in abundance by normal human gastric mucosal cells, by the ulcer associated cell lineage, a newly defined pathway of differentiation that appears in chronic inflammatory and ulcerative conditions, in hyperplastic polyps, and can also be induced in normal intestinal cell lines. They are secreted into the viscoelastic layer overlying the mucosa, and are also found in the neuroendocrine granules of gut endocrine cells. Whatever their perceived function in animals,[11] what can be the function of these abundantly expressed peptides in humans?

Curiously, a clue comes from the evolutionary conservation of these trefoil peptides. In *Xenopus laevis* skin, which contains abundant mucous glands, the trefoil peptide spasmolysin is found.[23] In rat intestinal goblet cells, a newly reported trefoil peptide has been detected – intestinal trefoil factor (ITF)[24]; rat goblet cells contain ITF peptide[24] and ITF mRNA transcripts[25]; a human form of the peptide also exists.[26] The mucin genes are encoded by a gene family of several members: MUC1 is expressed in breast, stomach, salivary gland, and the ulcer associated cell line, tissues where of course pS2 has been detected. Thus pS2, and possibly also hSP, follows MUCl expression. The intestine has other MUC genes, 2 and 3; ITF is found in the small intestine. Whereas the current evidence indicates that pS2 and hSP are orthodox regulatory peptides, a testable hypothesis is that each mucin type is cosecreted with its own trefoil peptide, and that trefoil peptides have an important role in the function of mucus.

Whatever their eventual designated role might be, it is clear that gastroenterologists are going to have a relation with trefoil peptides. Watch this shape.

NICHOLAS A WRIGHT

Department of Histopathology and
ICRF/RCS Histopathology Unit,
Royal Postgraduate Medical School/ICRF Laboratories,
London

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