The serpins (Serine Protease Inhibitors) are a superfamily of proteins which are involved in many important physiological processes such as blood coagulation and inflammation. The superfamily includes well-characterised inhibitors such as α1-antitrypsin and antithrombin III [1,2] as well as the non-inhibitory proteins ovalbumin and angiotensinogen. A sub-family of ovalbumin-like serpins [3] includes four human proteins: PAI-2, leukocyte elastase inhibitor, placental thrombin inhibitor, and the squamous cell carcinoma antigen (SCCA). SCCA is a tumour-associated antigen first detected in squamous cell carcinoma tissue of the uterine cervix [4]. The serum levels of SCCA are believed to reflect the degree of metastasis of cervical carcinomas, and a role for SCCA in tumour development has been proposed [5]. We have recently found a further ov-serpin gene, leupin which has 92% sequence identity to SCCA (Barnes & Worrall, unpublished data). The two genes differ greatly in their reactive loop sequences, with leupin having a leucine residue at its predicted P1 position where SCCA has a serine, suggesting that they may inhibit different target proteases.

Leupin was initially identified by screening genomic DNA using PCR to amplify sequences between 2 conserved regions of known serpins. Since then, the full length gene has been cloned from HeLa cells and both strands have been sequenced. An alignment of the SCCA and leupin reactive site loop sequences is shown below.

[Sequence alignment]

Fig. 1. Protein sequence alignment of the reactive site loops of Leupin with SCCA. Sequences given correspond to amino acids 342–366 of the 390aa proteins. Specific (antisense) primers to this region of either genes were used with a primer specific to the 5' region of both genes for RT-PCR expression studies.

Expression of SCCA and leupin was initially studied using Southern blotting techniques with specific probes. However, due to the high degree of similarity between the two sequences, crossreactivity of the probes occurred suggesting that previous expression studies of SCCA using antibodies and nucleotide hybridisation will have also detected leupin. To overcome this, we have designed oligonucleotides which are specific to the corresponding reactive loops of leupin and SCCA (Fig. 1).

RNA was isolated from a range of human cell lines and tissues and RT-PCR was performed using the specific oligonucleotide primers to detect and compare expression of SCCA and leupin.

Biochemical studies on SCCA have demonstrated that it can be separated into two groups, an acidic SCCA with an isoelectric point of <6.25, and a neutral SCCA with an isoelectric point of ≥6.25 [6]. We have detected leupin expression in HeLa cells, SKGIIa cells, and human placenta, all of which also express SCCA. However the levels of expression differ with leupin showing higher expression in placenta and HeLa cells (Fig 2).

The predicted molecular weight of leupin is 44,857 and its predicted isoelectric point is 6.04 which is consistent with the acidic form of SCCA. The presence of leupin in the same cell types as SCCA may explain the heterogeneity seen previously with SCCA. Furthermore, SCCA has recently been shown to inhibit Cathepsin L [7]. This inhibitory ability may be a property of either SCCA or leupin since protein preparations would contain both of these serpins.

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