

CRT Licensing Opportunity



Migration Stimulating Factor (MSF)

- Potent motogenic and angiogenic factor with a unique role in cancer progression
- MSF function neutralising antibodies demonstrate potent activity in *in vitro* cell-based migration assays
- Diagnostic and prognostic value associated with determination of MSF expression profile

BIOLOGICAL THERAPEUTICS | *In Vitro* Proof-of-Principle

July 2009

Background

CRT Discovery Laboratories (CRTDL) has undertaken pre-clinical studies aimed at validating MSF as a novel therapeutic target by demonstrating the potential of anti-MSF antibodies to prevent angiogenesis and tumour cell invasion, in collaboration with investigators at the University of Dundee.

The Target: MSF

MSF is a 70 kDa genetically truncated isoform of fibronectin containing a unique intron-derived C-terminal sequence not present in any previously described full-length (250-280kDa) fibronectin isoforms. Unlike fibronectin, MSF is not an extracellular matrix component, but a soluble cytokine exhibiting a number of bioactivities of relevance to cancer development. These include stimulation of cell migration (target: carcinoma cells, stromal fibroblasts and vascular endothelial cells), hyaluronan synthesis (target: fibroblasts) and angiogenesis. MSF's bioactivities are mediated by its constituent IGD tripeptide motifs. Full-length fibronectins do not express MSF-like bioactivities, presumably due to steric hindrance (unpublished, 1-2).

MSF and Cancer

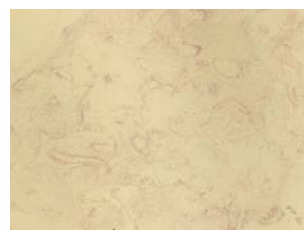
MSF is not significantly expressed by cells in healthy breast tissue, but is commonly expressed by mammary carcinoma cells, as well as by tumour-associated stromal fibroblasts and endothelial cells. Similar patterns of MSF expression have been demonstrated to be a feature of several common human cancers, including head and neck, colorectal, prostate, brain and skin tumours (unpublished, 1-2).

Inhibition of MSF functionality using specific function neutralising antibodies is anticipated to impede cancer progression as a consequence of the resultant (i) direct effects on tumour cell behaviour (i.e. inhibition of cell migration and hyaluronan synthesis), (ii) prevention of neo-angiogenesis, and (iii) the selective killing of newly formed blood vessels.

Stimulation of angiogenesis by MSF

MSF stimulates angiogenesis and cellular in-growth in a mouse subcutaneous sponge angiogenesis assay. Microscopic quantification of vessel density in the sponges shows a five-fold increase in vessel number on addition of 10ng/ml MSF compared to controls (Figure 1).

Injection of PBS



Injection of 10ng/ml of MSF

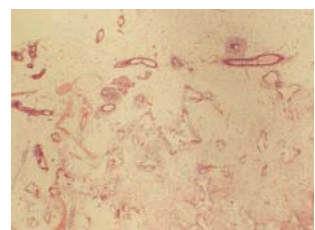


Figure 1: H & E staining of paraffin sections from sponges

Additional studies from investigators at the University of Dundee have shown MSF to be angiogenic in the following *in vivo* models: chick yolk sac membrane, subcutaneous implants in rat, subcutaneous implants in pig, and topical application to skin wounds in diabetic mice (unpublished, 1-2).

CRT Licensing Opportunity

Effect of MSF function-neutralising antibodies on cells

Motogenic activity of human recombinant eukaryotic MSF has been assessed *in vitro* in transmembrane and 3-D collagen assays. Migration of a range of endothelial cell types and breast tumour cell lines is stimulated by addition of MSF (1 pg/ml–100 ng/ml). MSF function neutralising antibody significantly inhibits MSF-stimulated migration in each case. These antibodies also inhibit the baseline migration and serum-stimulated migration of MSF-producer tumour cells.

Cell activation and sprouting cell aggregation has been modelled *in vitro* by plating vascular endothelial cells on the surface of a 3-dimensional collagen gel. These cells proliferate to form a homogeneous monolayer reminiscent of the monolayer lining the mature vessel lumen. Addition of an angiogenic factor to the cell culture results in a rapid induction of a second layer of cells displaying a spindle-shaped sprouting (angiogenic) morphology which form a network of multicellular aggregates. MSF function neutralising antibodies rapidly (within 24 hr) induce the disruption and death of such sprouting cell networks.

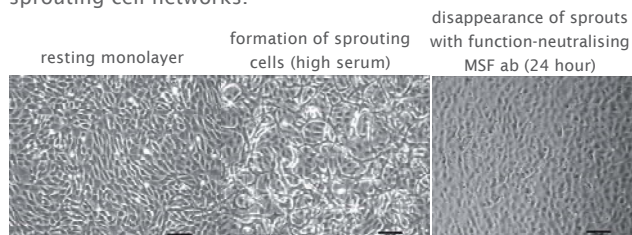


Figure 2: Selective killing of sprouting endothelial cells by MSF function neutralising antibodies

An anti-human MSF function neutralising antibody has also recently been reported to significantly suppress tumour growth and human tumour-related angiogenesis *in vivo* in an esophageal KYSE150 xenograft model without any obvious systemic side effects (3).

Diagnostic and prognostic value of MSF expression

MSF expression is low or negligible in the majority of normal adult tissues. In contrast, MSF is expressed in over 80% of all tumours examined. A statistically significant association between high MSF expression and poor survival has been observed in breast tumours (n=91) and oral tumours (n=54). These studies suggest that variations in the extent and cellular pattern of MSF expression may influence the clinical course of tumour progression and therefore convey diagnostic and prognostic information. Furthermore, assessment of MSF expression offers a theranostic strategy for the rational selection of patients for inclusion in therapeutic trials.

MSF and wound healing

Investigators at the University of Dundee have demonstrated MSF plays a role in acute wound healing as a consequence of its potent stimulation of cell migration, matrix remodelling and angiogenesis. MSF bioactivities are mimicked by small synthetic peptides containing the IGD amino acid sequence, as well as non-peptide IGD mimetics. Significantly, the bioactivities of MSF and its mimetics are critically modulated by the extracellular matrix, thereby affording the opportunity to fabricate bioengineered graft matrices containing MSF bioactives that are optimised to enhance wound healing in specific clinical indications, such as diabetic foot ulcers and pressure sores.

Intellectual Property

A portfolio of patent applications relating to MSF and its therapeutic applications is available for licensing, including WO99/31233 (granted US7351810, granted EP1042466, US, JP applications).

Commercial Opportunity

Inhibition of MSF is anticipated to be of considerable therapeutic value in the treatment of a range of cancers. CRT is seeking a commercial partner for further development of MSF therapeutics for oncology and other potential applications under exclusive licence or via collaboration.

References

1. Schor SL *et al.* Migration-Stimulatory Factor: A genetically truncated onco-fetal fibronectin isoform expressed by carcinoma and tumour-associated stromal cells. *Cancer Res.* 2003, **63**:8827-8836.
2. Schor SL and Schor AM. Review: Tumour-stroma interactions. Phenotypic and genetic alternations in mammary stroma: implications for tumour progression. *Breast Cancer Res.* 2001, **3**:373-379.
3. Hu H *et al.* Antibody library-based tumour endothelial cells surface proteomic functional screen reveals migration-stimulatory factor as an anti-angiogenic target. *Mol. Cell. Proteomics.* 2009, **8**:816-826.

Contact: Tanya Moore, tmoore@CancerTechnology.com

Ph: +44 (0)207 269 3640