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28

Macrophage Stimulating Protein (MSP) Promotes Tubular Regeneration And CD133+ Renal Progenitor Cell Differentiation After Kidney Ischemia-Reperfusion Injury (IRI)

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BACKGROUND

Delayed graft function (DGF) is an early complication of kidney transplantation (KT) mainly due to ischemia-reperfusion injury (IRI) and usually defined as the need for dialysis in the first week after transplantation.

The clinical relevance of DGF is due to its association with an increased risk of acute rejection, susceptibility to the nephrotoxic insult of calcineurin inhibitors and premature loss of graft function (chronic allograft nephropathy).

MSP is a plasminogen-related growth factor able to induce tissue regeneration and cell proliferation after binding to its specific tyrosine-kinase receptor named RON and located on the surface of different cell types including kidney tubular epithelial cells.

AIMS OF THE STUDY

The aims of this study were:
-to explore the role of MSP as potential biomarker of DGF or immediate graft function (IGF);
-to define the potential role of the MSP-RON pathway in kidney regeneration after IRI in the setting of DGF;
-to study *in vitro* the regenerative effect of MSP on tubular epithelial cells (TEC) cultured in hypoxia;
-to study *in vitro* the proliferative and differentiative effect of MSP on CD133+ renal resident progenitor cells.

METHODS

Plasma and urine levels of MSP (uMSP from R&D Systems, Minneapolis, MN) were evaluated by ELISA before and the first week after KT. Data were correlated with plasma NGAL (pNGAL from Alere, San Diego, CA), a known biomarker of DGF.

Immunohistochemistry for MSP and RON was performed at different time points on kidneys of male Wistar rats subjected to renal IRI by renal pedicle clamping for 45 minutes.

In vitro, we evaluated the effects of MSP on TEC subjected to hypoxia (proliferation, apoptosis, cell polarity) and on the differentiation of CD133+ renal progenitor cells isolated from the cortex of human kidneys.

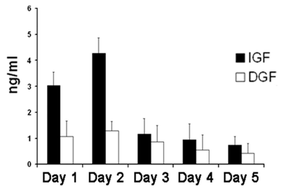

Figure 1:
Representative graph showing the comparison of the trends of urine levels of MSP (uMSP) between patients with Immediate Graft Function (IGF) or Delayed Graft Function (DGF). At day 2 after transplantation, uMSP levels were higher in patients with IGF in respect to patients with DGF

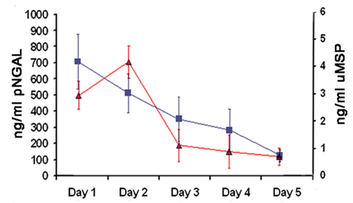

Figure 2:
Representative graph showing the comparison of the trends of urine levels of MSP (uMSP) and plasma levels of NGAL (pNGAL) in patients with Immediate Graft Function (IGF).

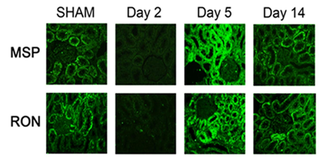

Figure 3:
Representative microscopy immunostaining for MSP and RON of kidney sections at different time-points after ischemia-reperfusion injury (IRI).

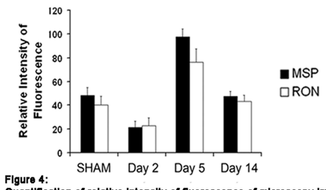

Figure 4:
Quantification of relative intensity of fluorescence of microscopy immunostaining for MSP and RON.

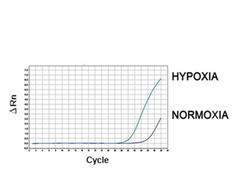

Figure 5:
Representative qRT-PCR analysis showing the increased mRNA expression of MSP in TECs coltured in hypoxia for 12h

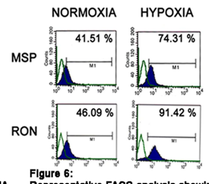

Figure 6:
Representative FACS analysis showing the increased protein expression of MSP and RON in TECs coltured in hypoxia for 12h

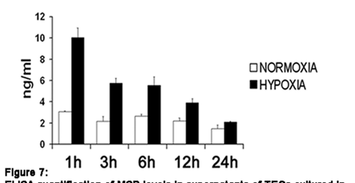

Figure 7:
ELISA quantification of MSP levels in supernatants of TECs cultured in hypoxia or normoxia at different time-points

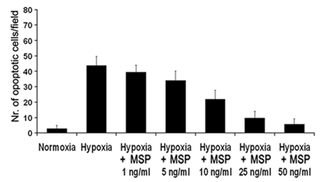

Figure 8:
TUNEL assay showing the anti-apoptotic effect of MSP on hypoxic TECs

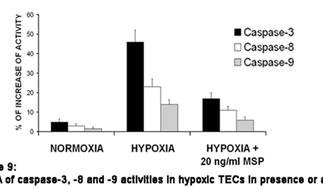

Figure 9:
ELISA of caspase-3, -8 and -9 activities in hypoxic TECs in presence or absence of MSP

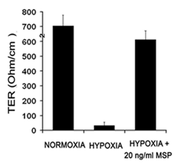

Figure 10:
Analysis of trans-epithelial resistance in TECs

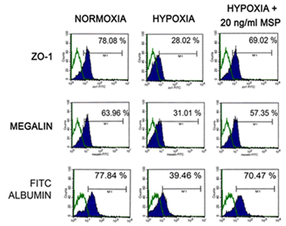

Figure 11:
Representative FACS analysis of ZO-1/Megalin expression and Albumin in TECs

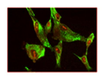

Figure 12:
Immunostaining for RON in CD133+ renal progenitor cells

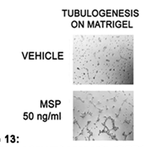

Figure 13:
MSP induced morphogenesis of CD133+ renal progenitor cells

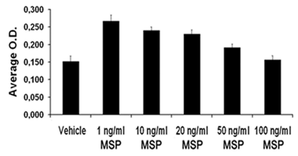

Figure 14:
BrdU assay of CD133+ renal progenitor cells in presence or absence of increasing doses of MSP

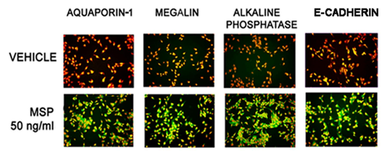

Figure 15:
Immunostaining for the epithelial/tubular markers expressed by CD133+ renal progenitor cells

RESULTS

At day 2 after transplantation, urine levels of MSP (uMSP) were higher in patients with IGF in respect to patients with DGF (IGF 4.24±0.51 ng/ml; DGF 1.28±0.60 ng/ml, Fig. 1). Moreover, in IGF patients the increase of uMSP levels peaked at day 2 in concomitance to the decrease of pNGAL (Fig. 2), indicating a prompt recovery of renal function.
In the experimental model of renal IRI in rats, a significant up-regulation of MSP and its receptor RON was observed in tubular epithelial cells in the regenerative phase after injury (day 5 after IRI, Fig. 3-4). These results suggest a putative autocrine release of MSP from regenerating tubular cells.
To confirm this hypothesis, we observed the mRNA/protein increased expression (Fig. 5-6) and release of MSP (Fig. 7) from TEC cultured *in vitro* under hypoxia. In hypoxic tubular cells, MSP induced proliferation (not shown) and resistance to apoptosis (Fig. 8) through the inhibition of both death receptor (Fas) and mitochondrial (Bcl-XL/Bcl-2) (not shown) apoptotic pathways and of caspase-3, -8 and -9 activation (Fig. 9).
MSP preserved also cell polarity through the maintenance of trans-epithelial resistance (Fig. 10), of megalin/ZO-1 expression and of internalization of albumin (Fig. 11).
In addition, we detected the expression of the RON receptor on CD133+ renal progenitor cells (Fig. 12) isolated from adult human kidneys. In presence of MSP, CD133+ proliferated with a scattering and branching morphogenesis (Fig. 13) and differentiated into mature epithelial cells acquiring cell polarity (Fig. 14) and a tubular-like-phenotype confirmed by the expression of aquaporin-1, megalin, alkaline phosphatase and E-cadherin (Fig. 15).

CONCLUSIONS

The results of the present study suggest that:
-MSP promotes tubular regeneration following renal IRI;
-MSP induces CD133+ renal progenitor cell differentiation;
-MSP may be envisaged as potential therapeutic approach for DGF after kidney transplantation.
Further studies enrolling a greater number of kidney transplanted patients are needed to define the role of urine MSP as potential biomarker of DGF/IGF.