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The role of macrophage migration inhibitory factor in vasoregression and neovascularization of the mouse retina

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Macrophage migration inhibitory factor (MIF) is a pleitropic proinflammatory cytokine that has proangiogenic properties. Its levels are increased in the vitreous and aqueous humor of patients with proliferative diabetic retinopathy. Given the potential of MIF to modulate angiogenesis, it is still unknown whether MIF plays a role in the development of diabetic retinopathy (DR), which is characterized by progressive vasoregression and pathological neovascularization. The aim of this study was to investigate the effect of MIF on vascular damage associated with diabetic retinopathy using Wildtype (WT) and MIF-knockout mice.

In the first part, we studied the role of MIF in physiological angiogenesis in the developing mouse retina. Retinal vascular development was analyzed by wholemount immunofluorescence at postnatal day 5 and 10. We observed that the development of the superficial vascular layer remained unaffected while the morphology of the vessels was slightly impaired in the deep capillary layer in the absence of MIF, indicating a proangiogenic effect of MIF in the development of the deep retinal vascular plexus.

In the second part, we investigated the effect of MIF on pathological retinal angiogenesis. The retinopathy of prematurity (ROP) model was used to study retinal vasoregression and neovascularization. Hyperoxia-induced vasoobliteration was analyzed by wholemount immunofluorescence. Intraretinal vessel regrowth was assessed by wholemount immunofluorescence and preretinal neovascularization was analyzed in retinal vertical sections after periodic acid-Schiff (PAS) staining in the hypoxic stage of the ROP model. Gene expression of proangiogenic and proinflammatory factors was measured in early-stage hypoxia (prior to neovascularization) by real-time PCR; at the same time point, recruitment of endothelial progenitor cells (EPCs) and microglial activation were analyzed with wholemount immunofluorescence. MIF-knockout mice developed larger areas of vascular obliteration (increased by 30%) in the hyperoxic condition, suggesting a protective effect of MIF in growth factor depletion-induced retinal vasoregression. In the hypoxic state, MIF-knockout mice had larger areas of vascular obliteration (increased by 49%), fewer sprouting tips (decreased by 27%), and reduced preretinal angiogenesis (decreased by 35%), indicating a proangiogenic effect of MIF in the hypoxia-induced retinal angiogenesis. MIF absence reduced the expression of erythropoietin (by 30%), TNF-α (by 70%), and ICAM-1 (by 50%), decreased the number of retinal EPCs by 37.5%, and inhibited microglial activation in the hypoxic condition.

In the third part, we explored whether MIF plays a role in diabetes-induced vasoregression. A streptozotocin-induced diabetic mouse model was used to study the effect of MIF in the diabetic condition. Both WT and MIF-knockout mice developed similar hyperglycemia. MIF expression was analyzed by immunofluorescence and Western blot. Microglial activation was assessed by whole-mount immunofluorescence. Six months after disease onset, vascular damage was assessed with retinal digest preparations and quantitative retinal morphometry. We found that the expression of MIF and microglial activation remained unaffected after six months of disease duration. Both diabetic WT and MIF-knockout mice developed increased acellular capillaries. Further, the absence of MIF did not affect diabetes-induced vascular damage, suggesting that MIF may not play a role in early stage diabetic retinopathy. Formation of advanced glycation end products (AGEs) is increased in diabetes and contributes to the pathogenesis of diabetic retinopathy. We studied whether exogenous Methylglyoxal (a major precursor of AGEs) induces CD74-positive microglial activation in the normoglycemic retina and whether absence of MIF inhibits this process. Methylglyoxal was injected intravitreally in both non-diabetic WT and MIF-knockout mice. We found that exogenous MG induced an increase of CD74-positive cells in both WT and MIF-knockout groups and that the absence of MIF

did not significantly change this increase, thus suggesting that CD74 may play a role in this response independent of MIF.

In conclusion, we found that MIF has proangiogenic and proinflammatory properties in the retinal neovascularization process and plays a protective role in oxygen-induced, but not diabetes-induced retinal vasoregression. The proangiogenic role of MIF in ischemia-induced retinal neovascularization may be associated with its influence on the expression of erythropoietin, EPC recruitment and inflammation. Therefore, MIF may serve as a potential therapeutic target in the pathological angiogenesis of diabetic retinopathy.