Impaired angiogenesis in double MMP-2 and MMP-9 deficient mice

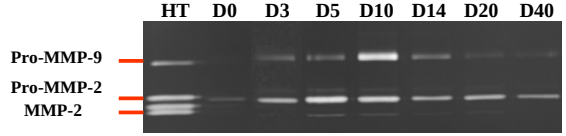
V. Masson [1] , V. Lambert[1] , L. Rodriguez de la Ballina[1], C. Munaut[1], B. Wielocks [3], M. Jost[1], Z. Werb[4], T. Itoh, C. Libert[3], J.-M. Rakic[2] , J.-M. Foidart[1] and A. Noël [1]

[1]Laboratory of Tumor and Development Biology, C.R.C.E., University of Liege, Belgium ; [2]Ophthalmology, University Hospital (CHU), Liege, Belgium;
[3]Department of Molecular Biology, University of Ghent, Belgium; [4]Department of Anatomy, University of California, San Francisco, CA

A- Choroidal neo-angiogenesis

MMP-2 and MMP-9 are produced in laser-induced lesions associated with choroidal neovascularization

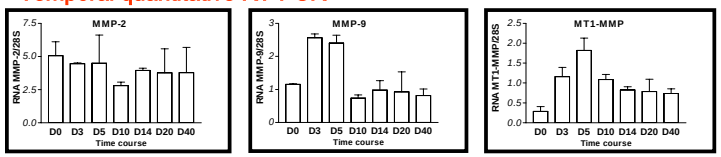
Temporal gelatin zymography



Gelatin zymography analysis of ocular posterior segments demonstrated that both MMP-2 and MMP-9 were increasingly produced during the early stages of CNV formation, with the appearance of active forms of MMP-2.
In situ zymography revealed a predominant gelatinase activity in the CNV area.

Different regulation of MMP-2 and MMP-9 expression

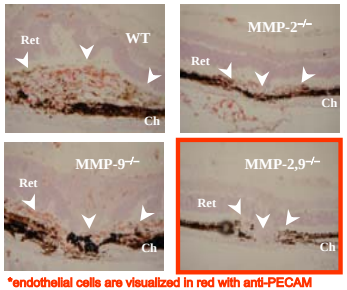
Temporal quantitative RT-PCR



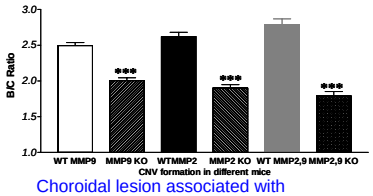
RT-PCR evaluation showed that MMP-9 expression was upregulated during early phases of CNV formation, while MMP-2 was constitutively expressed without any transcriptional modulation. Interestingly, MT1-MMP mRNA was concomitantly upregulated suggesting that the presence of active MMP2 forms was due to the expression and activity of its activator.

Severe inhibition of choroidal neovascularization in MMP-2,9 double deficient mice

CNV formation in single or double gene deficient mice*



*endothelial cells are visualized in red with anti-PECAM



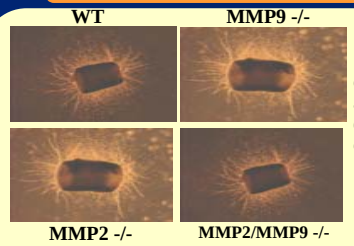
A significant reduction of the B/C ratio was observed in MMP-9 (33 %), MMP-2 (44%) and MMP-2,9 (56%) deficient mice compared to their corresponding WT (*p<0.001*).

Choroidal lesion associated with neovascularization was quantified by determining the B/C ratio between total lesion thickness ("B", maximal height lesion measured from the bottom of the choroid to the top of the neovascular area) to the thickness of adjacent normal choroid ("C").

ABSTRACT

In the present study, we addressed the specific functions of individual MMPs as anti- or pro-angiogenic mediators by assessing **the impact of single or combined MMP deficiencies in two in vivo models of pathological angiogenesis (malignant keratinocyte transplantation and laser-induced choroidal neoangiogenesis) and in an in vitro model of angiogenesis (the aortic ring assay).** The single deficiency of MMP2, MMP3,or MMP9 did not impair in vivo tumor invasion and vascularization. However, the cooperative effect of both gelatinases is demonstrated by the inhibition of tumor vascularization and growth **in double MMP2/MMP9 deficient mice**. Similarly, both the incidence and severity of choroidal neoangiogenesis induced by a laser burn were strongly attenuated in these double deficient mice. In sharp contrast, single or combined lack of gelatinases did not impair the in vitro spreading of capillaries from aortic rings, suggesting the importance of a cross talk between several host cells for the concerted MMP2 and MMP9- mediated formation of new blood vessels. Altogether, these data demonstrate that both MMP2 and MMP9 contribute to in vivo tumoral and choroidal neo angiogenesis.

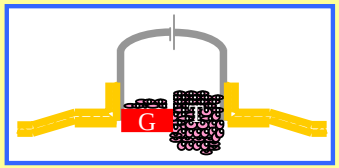
C- Aortic ring assay



The single or combined MMP2 and MMP9 deficiency did not affect vessel outgrowth in vitro.

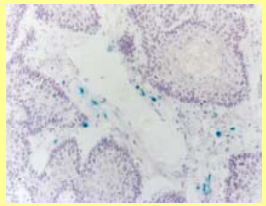
The expression of MMP9 by vascular cells was assessed by using the MMP9/LacZ. In these conditions, a b-galactosidase positive staining observed in microvessels spreading out identified the activity of MMP9 promoter in endothelial cells.

B- Tumoral angiogenesis



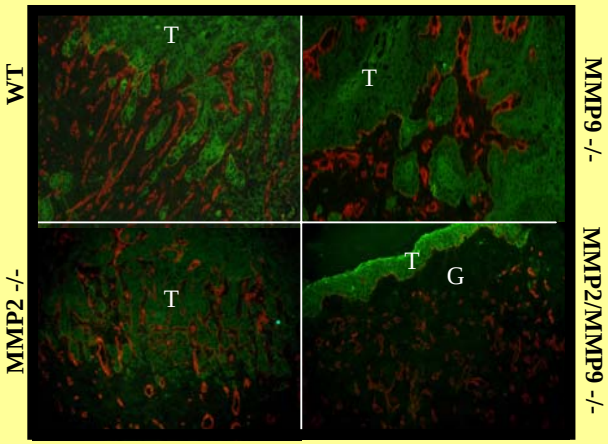
Transplantation chamber used for the grafting of transformed keratinocytes

G= collagen gel
T= tumor cells



Transplantation of malignant keratinocytes into transgenic mice expressing beta galactosidase under the control of MMP9 promoter. MMP9 is expressed at the tumor site by host cells

Malignant keratinocytes (labelled in green with an anti-keratin antibody) were cultured on a collagen gel and then transplanted into wild type (WT) or single or double KO mice. Vessels were labelled in red with an anti CD31 antibody.



While the single deficiency of MMP2 or MMP9 did not affect tumor invasion and vascularization, their combined deficiency impaired tumor progression.

The comparison between the in vitro aortic ring assay and the in vivo malignant keratinocyte angiogenic phenotype indicates clearly that MMP2 and MMP9 are not absolute prerequisites for collagen penetration by activated endothelial cells. The lack of in vivo penetration into the collagen gel therefore indicates that in the double MMP2/MMP9 -/- mice, the endothelial cells are not adequately stimulated. Adequate endothelial cell activation requires therefore a source of gelatinolysis that can be brought by MMP2 or MMP9 secreting cells.