Severe Inhibition of Choroidal Neovascularization in Mice With a Combined Deficiency of MMP-2 and MMP-9 Genes

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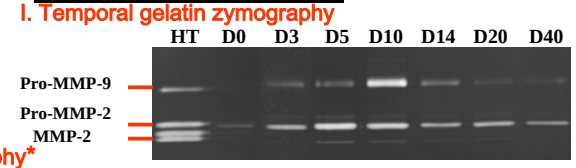
A- ABSTRACT

Previous studies have shown that the production of gelatinases (MMP-2 and MMP-9) belonging to the matrix metalloproteinase family is increased in human choroidal neovascularization (CNV) occurring during the exudative most aggressive form of age-related macular degeneration (AMD). To more precisely delineate the respective roles of MMP-2 and MMP-9 in choroidal neo-angiogenesis, we investigated their expression and activities in the course of laser-induced murine choroidal neovascularization,. This model was applied to single (MMP-9 KO, MMP-2 KO) or double (MMP-2,9 KO) deficient mice and to their corresponding wild-type (WT) controls.

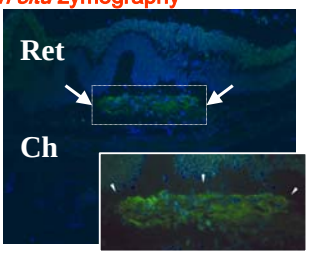
B- RESULTS

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

I. Temporal gelatin zymography



II. *In situ* zymography*

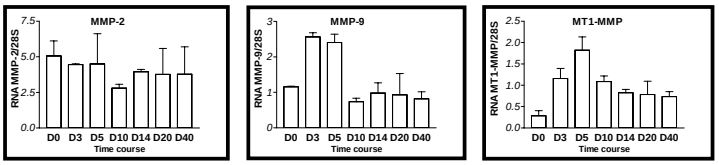


*Fluorescein-conjugated gelatin

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

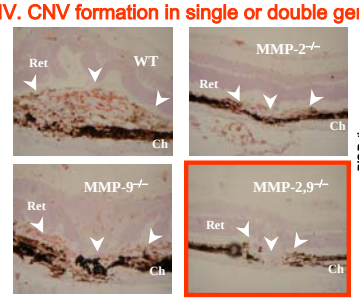
III. Temporal quantitative RT-PCR

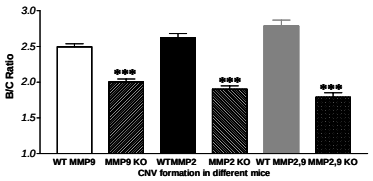


RT-PCR evaluation showed that MMP-9 expression was upregulated during early phases of CNV formation, while MMP-2 was constitutively expressed without any transcriptional modulation. Interestingly, MT1-MMP mRNA was concomitantly upregulated suggesting that the presence of active MMP2 forms was due to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

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

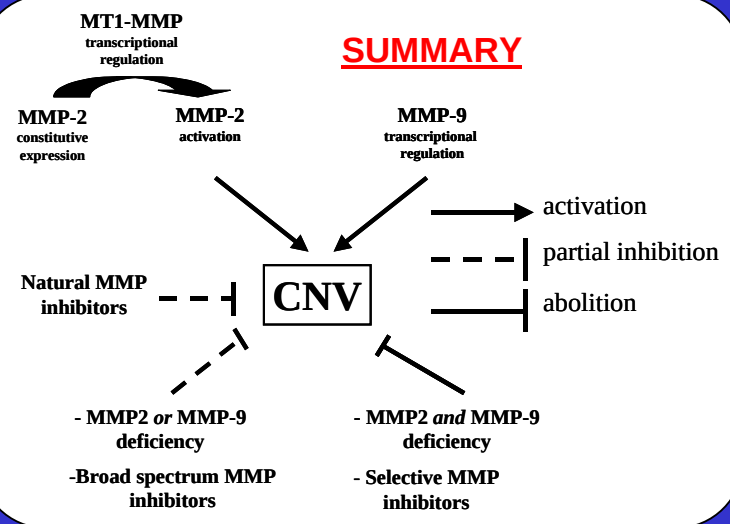
IV. CNV formation in single or double gene deficient mice*



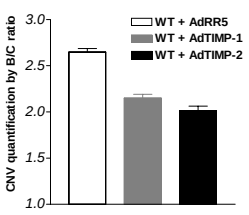


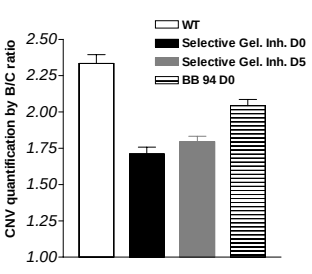
*endothelial cells are visualized in red with anti-PECAM

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"). 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 ($p<0.001$).



V. Results of MMP inhibition





In a second approach to assess whether MMPs contribute to CNV development, we first induced endogenous overexpression of TIMP-1 or TIMP-2 by adenoviral-mediated delivery in WT mice. Both TIMP-1 and TIMP-2 overexpression significantly reduced choroidal angiogenesis ($p<0.001$) compared to WT controls injected with control viruses (AdRR5). We then evaluated the effects of broad spectrum (BB-94) or more selective MMP inhibitors (Ro 28-2653 inhibiting preferentially MMP-2, MMP-9 and MT1-MMP) on CNV development by treating WT mice with daily systemic injections. Both inhibitors significantly reduced the CNV formation. However, Ro-28-2653 was significantly more efficient ($p<0.001$) than BB-94. Interestingly, selective MMP inhibition treatment started five days after laser induction also significantly inhibited the development of choroidal angiogenesis (40% inhibition).

C- CONCLUSIONS

- Both gelatinases are increasingly processed and concentrated in the region of CNV development.
- Choroidal pathological angiogenesis is nearly fully prevented in MMP-2/MMP-9 double deficient mice, while it is only partly impaired in the single MMP deficient mice.
- Choroidal angiogenesis was strongly inhibited in mice treated with a selective gelatinase/MT1-MMP synthetic inhibitor.

MMP-9 expression in the course of CNV development is transcriptionally regulated, while MMP-2 is regulated by zymogen activation as the result of an overexpression of its main activator, MT1-MMP.

- A synthetic inhibitor interacting preferentially with MMP-2, MMP-9 and MT1-MMP inhibits more efficiently choroidal neovascularization than a broad spectrum synthetic inhibitor.

- MMP inhibitors might have a potential interest for neovascular regression. This is a crucial question in clinic since most patients affected by the exudative form of AMD present at a late stage where the neovascular membrane is already developed.

In addition, the observation of a synergy between MMP-2 and MMP-9 might be of interest for other pathological conditions associated with angiogenesis such as tumoral development