UNVEILING DPP9 AND CARD8 EXPRESSION IN PRIMARY HUMAN DENDRITIC CELLS

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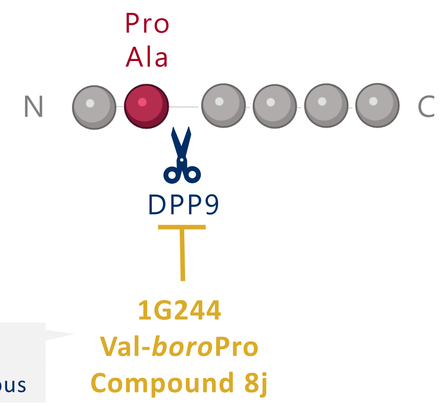
BACKGROUND

Enzymatic activity

DPP9 cleaves a dipeptide from the N-terminus of its substrates

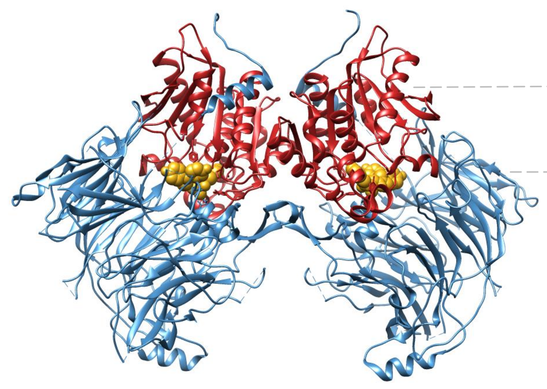


DPP8/9 inhibitors
DPP8 & 9 are highly homologous

1G244
Val-*boro*Pro
Compound 8j

Dipeptidyl peptidase 9

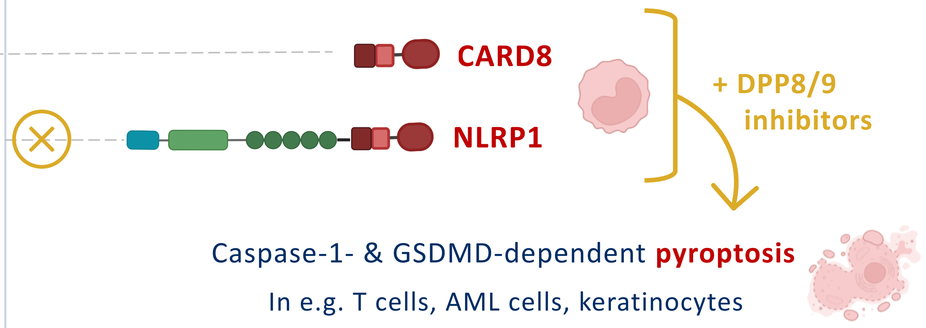
DPP9



DPP9 dimer with 1G244 (PDB-ID: 6EOR)

Binding partner

DPP9 represses inflammasome activation by interacting with 2 inflammasome-forming proteins



AIMS

Measure DPP8/9 activity in primary human myeloid blood cells

Investigate DPP9 and CARD8 subcellular expression

METHODS

DPP8/9 activity

- DPP activity was measured using a fluorometric enzymatic activity assay in monocytes, Mϕ and moDC
- The enzymatic activity contributed by DPP8/9 is defined as the proportion of activity inhibited by **1G244**

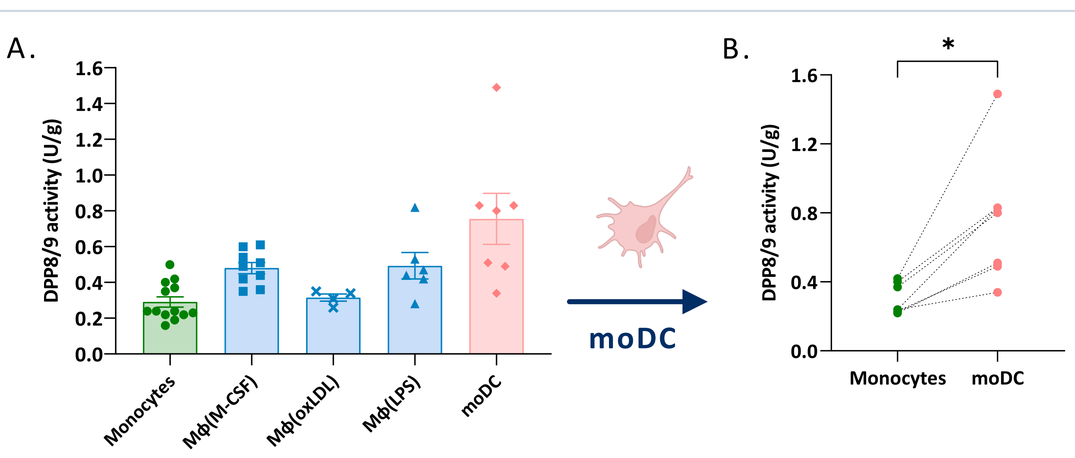
Cell culture

- PBMCs were isolated from peripheral blood of healthy volunteers
- Monocytes were enriched from the PBMC fraction via CD14+ positive magnetic selection using CD14 microbeads (Miltenyi)
- Differentiation of CD14+ cells into monocytes, macrophages (Mϕ) and monocyte-derived dendritic cells (moDC)

Immunofluorescence

- Cells were stained for DPP9 and/or **CARD8**
- Subcellular localisation of DPP9 and CARD8 were visualized using confocal microscopy

RESULTS



DPP8/9 activity in primary human myeloid cells. (A) DPP8/9 activity was determined in cell lysates using Gly-Pro-4-Me-β-NA at pH 8,3. Data are represented as mean ± SEM (n = 4-13). **(B)** DPP8/9 activity increased upon monocyte-to-moDC differentiation in cells of all donors. Wilcoxon matched-pairs signed rank test, * $p < 0.05$.

DPP9 and CARD8 subcellular localization in primary myeloid cells. DPP9 was localized in the cytoplasm and, strikingly, also in the nucleus of monocytes, Mϕ, and moDC. Z-stack images were captured to further analyze nuclear DPP9 expression. Of note, the subcellular expression of DPP9 was variable between cells of the same donor. In addition, CARD8 was located in the cytoplasm of all cell types and the overlap between DPP9 and CARD8 staining was most pronounced in moDC. CARD8 expression is indicated in red, DPP9 in green and nuclei are stained blue. Magnification: x40. Scale bar: 10 µm.

CONCLUSIONS

First study to show that DPP9 may play a significant role in primary human moDC

- DPP8/9 activity was upregulated upon monocyte-to-Mϕ [5] & monocyte-to-moDC differentiation
- After immunofluorescent staining, areas of overlap between DPP9 and CARD8 was mostly observed in moDC
- DPP9 subcellular expression was variable between cells of the same donor
- Strong nuclear DPP9 expression may indicate an undiscovered nuclear function of DPP9 in human myeloid cells

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Research Foundation Flanders
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This work received support from the Flanders Research Foundation (FWO, predoctoral fellowship JDL and YVR). BioRender was used to create images.

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