Targeting MICA with therapeutic antibodies for the treatment of cancer

Authors

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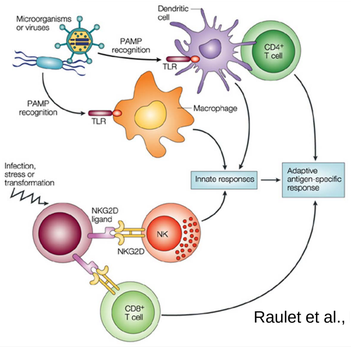
Introduction

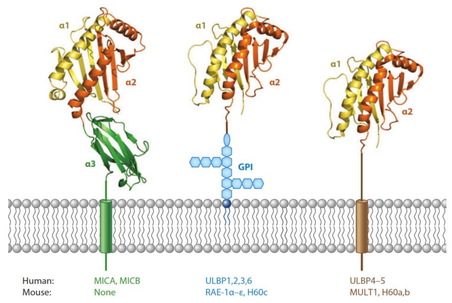
MICA and MICB, along with ULPBs, are ligands for the activating receptor NKG2D expressed on NK cells and subsets of T cells in Human. NKG2D ligands are induced by cellular stress and pathogen infections. Their expression is tightly regulated by complex mechanisms both at the mRNA and protein levels. In the case of MICA and MICB, more than 65 and 30 alleles respectively were described with different properties regarding to their cellular location adding to the complexity of this recognition system. Nevertheless, as markers of cellular stress, in particular in tumorigenesis, MICA and the closely related MICB proteins are candidates of choice to be targeted by a cytotoxic therapeutic antibody.

We have generated a panel of anti-MICA mAbs with diverse functional properties. Ongoing work aims to choose the best candidate for humanization and further clinical development.

NKG2D-Ligands



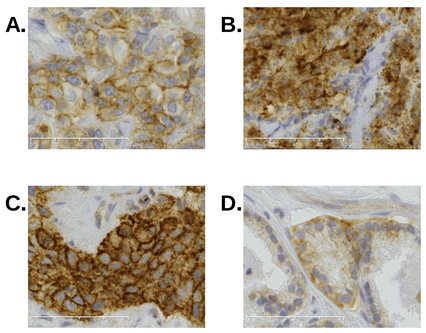
Raulet et al., Nat. Rev. Immunol. (2003)



Raulet et al., Annu Rev Immunol. (2013)

Results

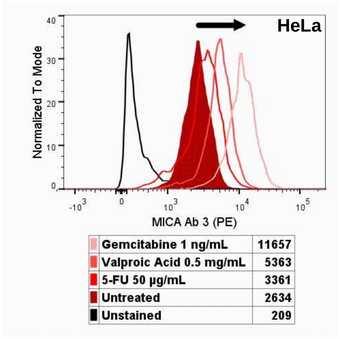
MICA is a tumoral antigen



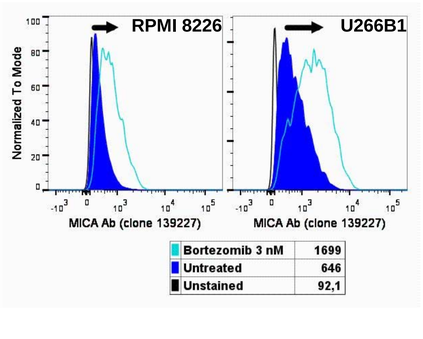
Tissue	Positive/ Total cases	% Positive
A. Breast Cancer	24/29	83%
B. Colo-rectal Cancer	17/28	61%
C. Lung Cancer	18/28	64%
D. Prostate Gland	3/5	-

- Representative paraffin-embedded sections of indicated cancer were stained with 1 µg/mL anti-MICA/B BAM01). Isotype control or secondary antibody did not yield any specific signal (not shown). The table recapitulates results obtained on several sections. 72 types of normal tissues were tested for MICA/B expression and no expression could be detected except in the parathyroid gland.

Chemotherapeutic agents upregulate MICA expression



Gemcitabine 1 ng/mL	11657
Valproic Acid 0.5 mg/mL	5363
5-FU 50 µg/mL	3361
Untreated	2634
Unstained	209



Bortezomib 3 nM	1699
Untreated	646
Unstained	92,1

- MICA expression was measured with indicated anti-MICA mAb by flow cytometry after 48h *in vitro* treatment with indicated chemotherapeutic agents. Values indicate Median Fluorescence Intensity in each condition.

Characterization of new anti-MICA/B monoclonal antibodies

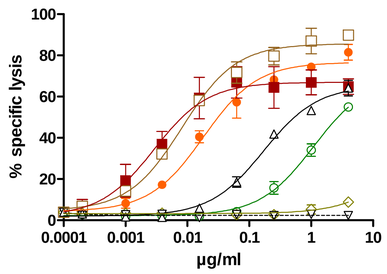
Binding	MICA Ab 1	MICA Ab 2	MICA Ab 3	MICA Ab 4	MICA Ab 5
KD (nM) MICA*01 (SPR)	8.9	NM	6.5	40	11
KD (nM) MICA*08 (SPR)	150	3.1	0.5	90	ND
EC50 (ng/mL) MICA*01 (FACS)	329	2286	1309	215	718
EC50 (ng/mL) MICA*04 (FACS)	2292	136	41	51	158
EC50 (ng/mL) MICA*07 (FACS)	342	3149	177	566	NM
EC50 (ng/mL) MICA*08 (FACS)	2987	127	57	81	NM
MICB (FACS, SPR)	Yes	No	Yes	No	Low
ULBP1, -2, -3 (SPR)	No	No	No	No	No
NHP MaFaMIC (FACS)	Yes	Yes	Yes	Yes	Yes

- Anti-MICA/B of high affinity were generated. Examples of monovalent affinities measured by surface plasmon resonance (SPR) on recombinant soluble MICA *01-BirA or MICA*08-His proteins, as well as examples of EC50 values measured by flow cytometry (FACS) on MICA transfected C1R cells are indicated. ND: Not Done. NM: Not Measurable
- Anti-MICA/B Abs 1, 3 and 5 cross-react on MICB (SPR and FACS). None of these Abs cross-react on ULBP-1, -2 and -3 (SPR) but they all cross-react on MIC sequences from Macaca fascicularis transfected in to BaF/3 cells (FACS).

Anti-MICA/B mAbs *in vitro* efficacy

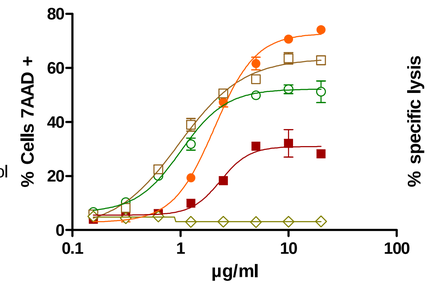
ADCC

KHYG-1-hCD16 vs. C1R-MICA*08



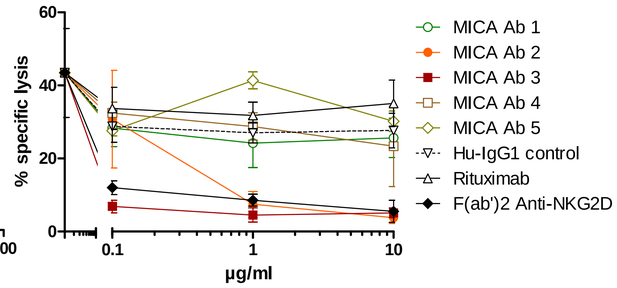
CDC

human complement vs. Raji-MICA*01



NKG2D blocking capacity

NK92 vs. Raji-MICA*08

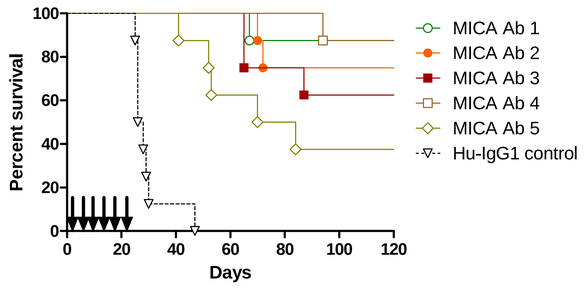


- ADCC: 4h- ^{51}Chromium Release Assay with indicated cell lines at an Effector/Target ratio of 20/1.
- CDC: Ab-coated Cells were incubated for 3h at 37°C with 20% human serum complement. Dead cells were measured by flow cytometry as 7-AAD-positive cells.
- NKG2D blocking capacity: 4h- ^{51}Chromium Release Assay with indicated cell lines at an Effector/Target ratio of 10/1

Mean + SD of experimental triplicates are shown on graphs. Filled symbols = NKG2D blocking Ab.

Anti-MICA/B mAbs *in vivo* efficacy in xenograft models

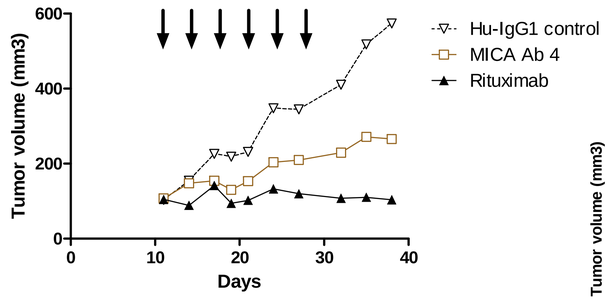
Preventive setting (IV model)

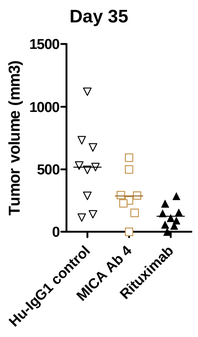


- 15 millions Raji-MICA*01 were injected I.V. in NOD-SCID mice. Mice were treated twice a week for 3 weeks with 300 µg of the indicated Ab (Black arrows).

N=8 mice per group.

Curative setting (SC model)





- 15 millions Raji-MICA*01 were injected S.C. in NOD-SCID mice with matrigel. Tumors were allowed to grow then mice were randomized and were treated twice a week for 3 weeks with 300 µg of the indicated Ab (Black arrows).

N=9 mice per group, median tumor volume.

Conclusions

MICA can be targeted by therapeutic antibodies in cancer

- MICA is specifically expressed on many tumors and not in normal tissues
- Chemotherapeutic agents upregulate MICA expression
- Newly generated anti-MICA mAb display high affinities, are pan-alleles and can mediate direct cytotoxic effect through ADCC and/or CDC and demonstrate *in vivo* efficacy
- Additional modes of action of anti-MICA mAb are currently investigated including:
  - ✓ Neutralization of soluble MICA (direct effect or inhibition of shedding)
  - ✓ Restoration of NKG2D expression and function
- Associated biomarker strategy: MICA expression on tumor, sMICA in serum

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