

Characterization of the immune profile of cancer stem cells isolated from human glioblastoma multiforme.

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“Stem cell like” and tumors

- **Cancer is constituted of a heterogeneous population of cells differing in morphology, marker expression and proliferation capacity; this heterogeneity can be explained by genetic, epigenetic and micro-environmental differences.**
- **This might occur due to the fact that a tumor is hierarchically organized, with its own stem cell compartment (CSCs), though this issue is still controversial.**
- **CSCs can be defined as:**
 - 1) **self-renewing cells;**
 - 2) **cells that give rise to the variety of differentiated cells found in the malignancies (multipotency);**
 - 3) **cells able to generate a phenocopy of the original malignancy in immunocompromised mice (tumorigenic ability).**

Tumor associated antigens in GBM:

Overexpression of **EGFR**, and its mutant form **EGFR ν III** (30-60% GBM).

gp100 and **TRP2** in 64 and 21 % of GBM cases, respectively (Saikali S., *J Neurocol*, 2007).

IL-13R α 2: overexpression of interleukin 4 (IL4)-independent binding sites for IL-13 (IL-13R α 2) in situ. The gene for IL13R alpha maps on chromosome X and this antigen is categorized as a cancer/testis antigen (Debinski W., *Mol Med*, 2000).

EphA2: tyrosine kinase receptor for ephrins; involved in the regulation of development processes particularly in the CNS. This molecule is over-expressed in GBM and other solid tumors, such as breast and pancreatic cancers.

(Wykosky J., *Clin Cancer Res*, 2008).

SOX2: Sox1-3 genes are highly expressed in CNS during embryonic development and are down-regulated as neural cells starts to differentiate. T lymphocytes specific for SOX2 and for GBM cells were generated by *in vitro* stimulation with SOX2-derived epitope of PBMC from healthy donors (Schmitz M., *Brit. J. Cancer*, 2007).

Aims

- **Immunological characterization of CSCs isolated from GBM patients;**
 - **Definition of MHC class I and II expression by CSCs;**
 - **Definition of NKG2D ligand expression by CSCs;**
- **Definition of T lymphocyte recognized TAAs expressed by CSCs;**
- **Assessment whether CSCs can represent target cells for new immunotherapeutic treatment for GBM patients.**

Phenotype analysis of the expression of i) MHC class I and class II molecules, ii) NKG2DLs (MICA/B, ULBP1-4) by GBM CSCs.

Modulation of the expression of MHC molecules by IFN- γ treatment of GBM-CSC lines.

CSC line	IFN- γ	MHC I	MHC II (DR)
070112 GI	-	1	1
	+	58	3
070112 GD	-	1	1
	+	17	5
070104 GD	-	1	1
	+	12	2
0627 GI	-	30	2
	+	44	3
0627 GD	-	3	1
	+	9	1
030616 GD	-	3	1
	+	15	1
050115 GD	-	2	1
	+	12	1

GI: growth factor independent;

GD: growth factor dependent;

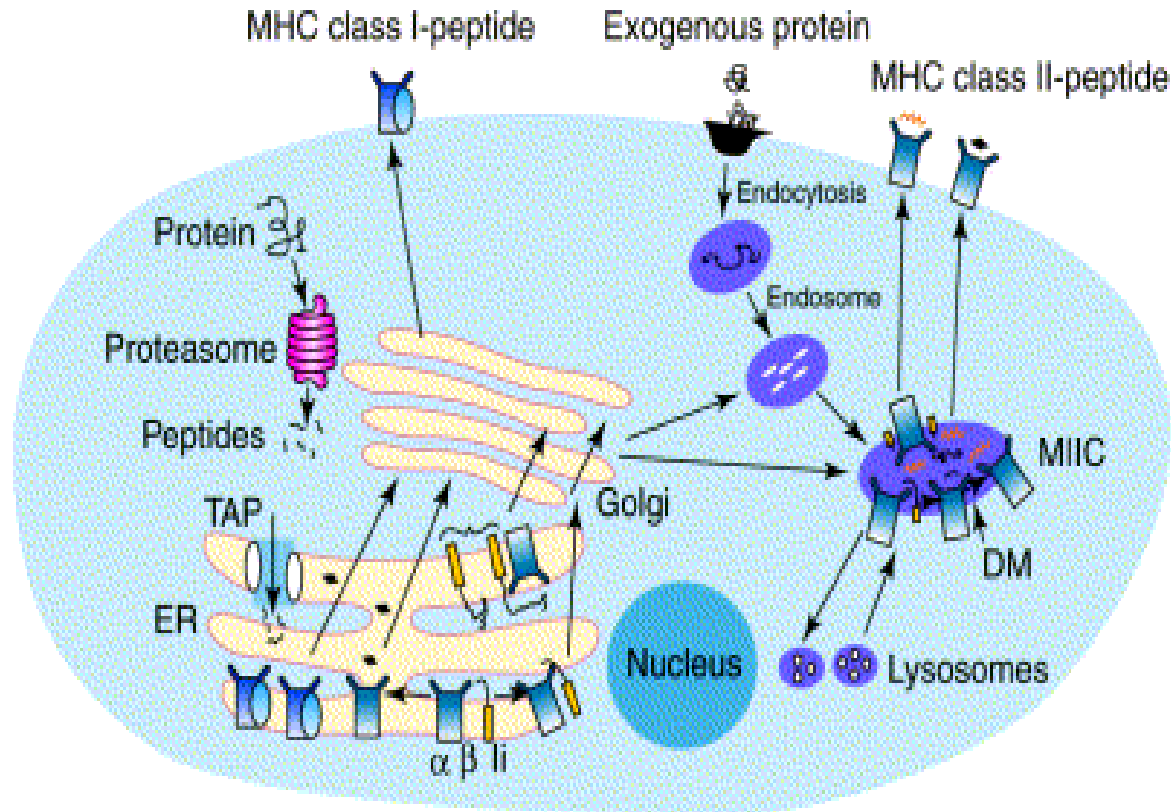
MRFI: ratio between the MFI of cells stained with the selected mAb and that of cells stained with isotype-matched control mouse immunoglobulins;

Conclusion: Efficient up-regulation of MHC class I and weak modulation of MHC class II molecules by CSC lines was achieved by IFN- γ treatment.

MHC class I and II antigen processing and presentation.

(a) MHC class I antigen processing

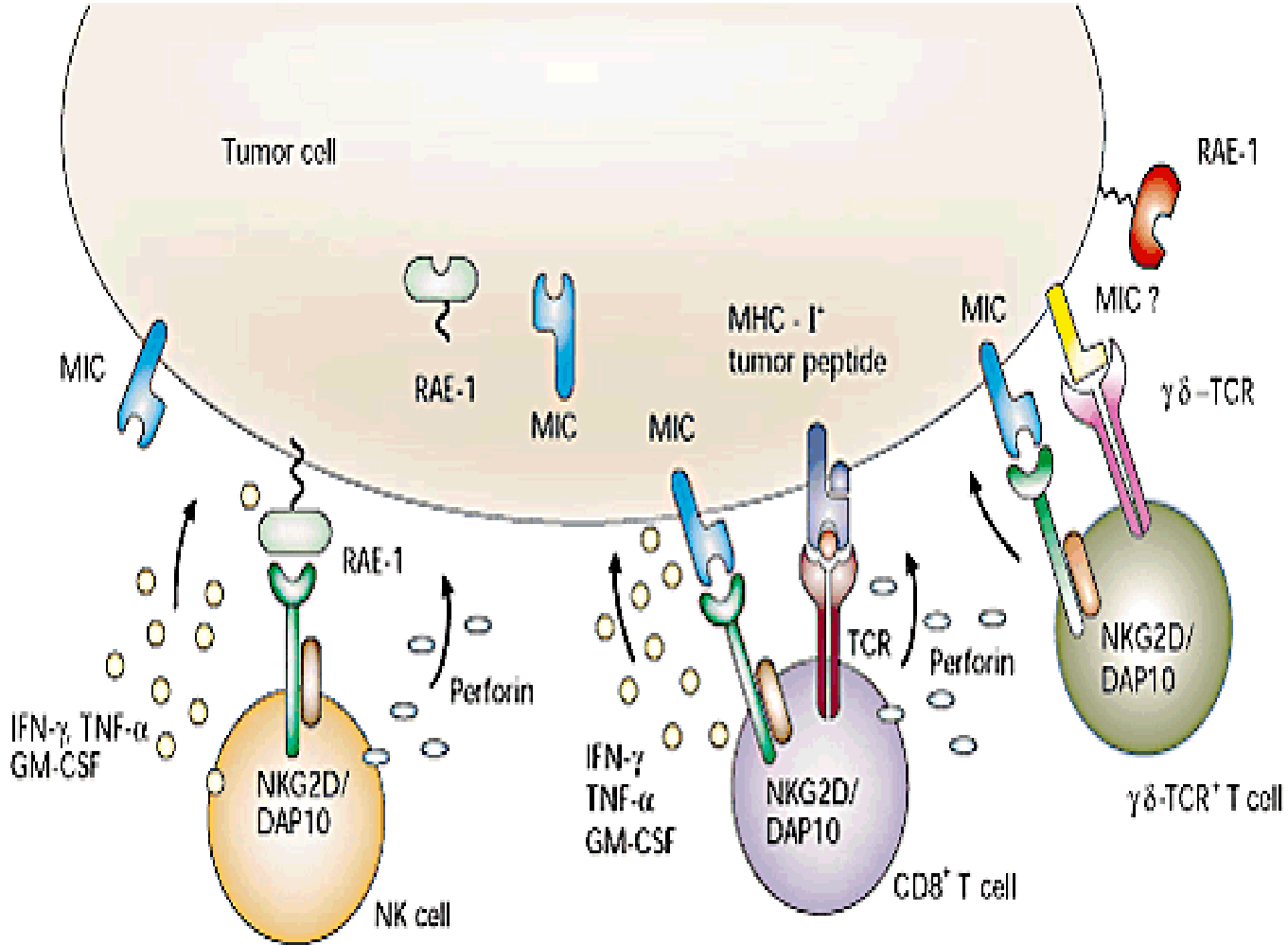
(b) MHC class II antigen processing



TRENDS in Immunology

(a) The MHC class I pathway favors presentation of endogenous proteins. Proteins are degraded by proteasomes to generate an array of peptides that enter endoplasmic reticulum (ER), where MHC class I-peptide complexes form, and then transport to the cell surface for recognition by CD8⁺ T cells. (b) The MHC class II pathway preferentially presents exogenous proteins. The key stage of this pathway is the trafficking of both exogenous proteins and MHC class II molecules to the same compartment, the MHC class II compartment (MIIC).

NKG2D cooperates to the NK, NKT or T (TNK) cell mediated-anti-tumor immune responses.



Expression of TAAs by GBM CSCs

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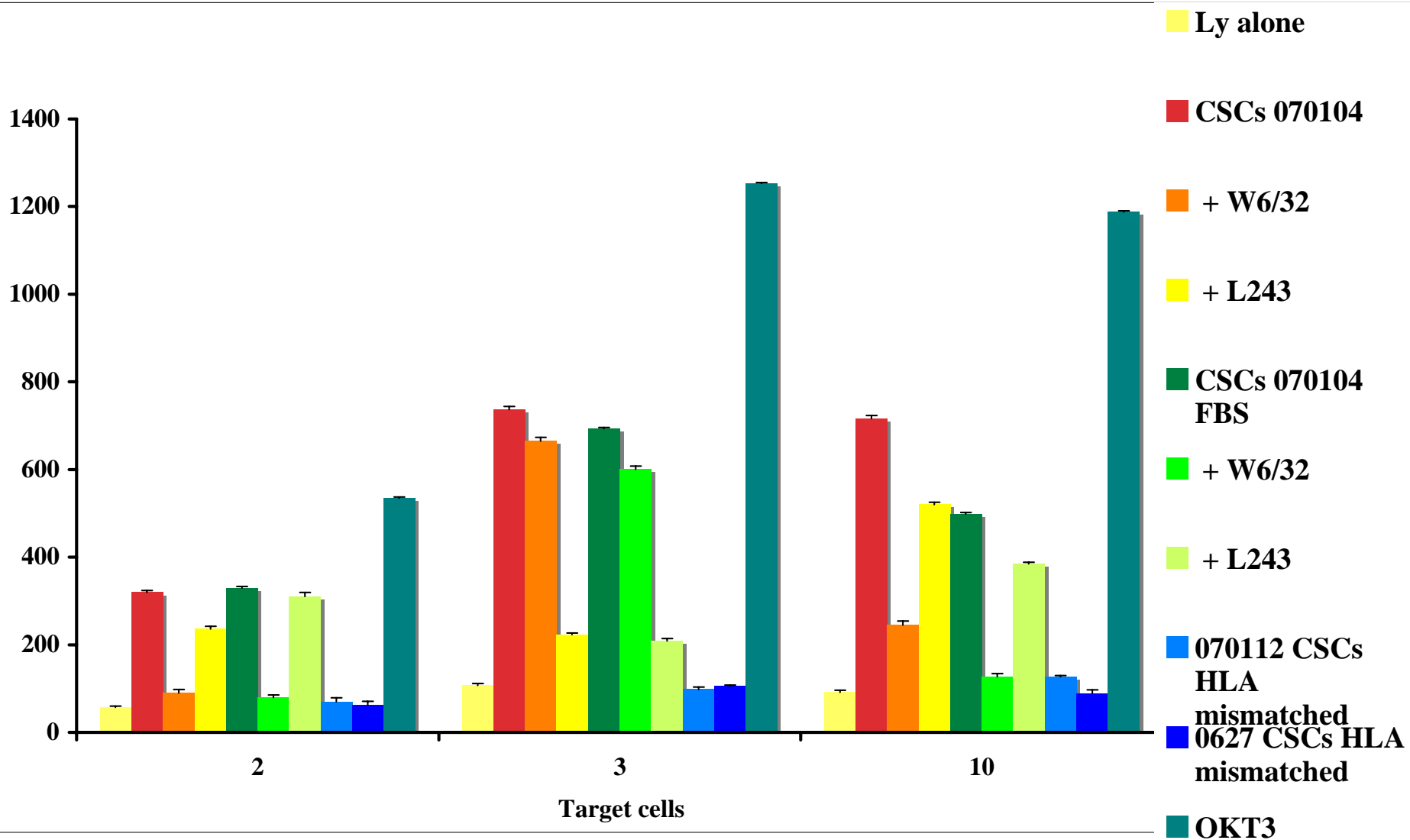
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Results: Efficient expression of Survivin and COA-1 was commonly detected in GBM CSCs.

- **Assessment whether CSCs can represent target cells for T cell-mediated immune responses in GBM patients.**

CSCs can elicit anti-GBM specific immune responses.



T cells expressed homogeneously CD3 and CD45RA; they were mostly CD8⁺ (cultures n. 2 and 10) or CD4⁺ (culture n. 3) and CD127⁺, CD27⁻ and CCR7⁻.

Conclusions (I)

- **MHC or NKG2DL molecules were weakly or not expressed by GBM-CSCs;**
- **Efficient modulation by IFN- γ or IFN- α treatment of GBM-CSCs was achieved only for MHC class I and not for MHC class II molecules;**
- **APC machinery expression was defective in GBM CSCs;**
- **Partial up-modulation of APC machinery molecules was achieved by IFN (α or γ) treatment of CSCS;**
- **Efficient expression of the TAA Survivin, COA-1 and SOX2 but not of MAGE and Gp100 was detected in GBM-CSC lines. Weak expression of NY-ESO occurred in a few CSC lines. A wider expression array of TAAs by CSCs need to be evaluated.**

Conclusions (II)

- **T cells, either CD8⁺ or CD4⁺, exerting GBM CSC-specific recognition (evaluated by IFN- γ release) can be elicited by *in vitro* stimulation of PBMCs with autologous CSCs isolated from one GBM patient (070104);**
- **CD8⁺ T lymphocytes isolated from one GBM patient (070104) exerted specific cytotoxic activity against autologous CSCs as assessed by cytofluorimetric analysis of CD107a mobilization;**
- **TAA recognized by CSC-specific T lymphocytes need to be molecularly determined;**
- **The validation of CSCs as efficient source of TAAs to elicit anti-GBM immune responses will be determined in a large number of patients.**

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