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Tumor Heterogeneity Uncovered by HLA-G Isoforms Expression

Diana Tronik-Le Roux, Jérôme Verine, Alix Jacquier, Raluca Stanciu,
Julie Renard, Chantal Schenowitz, François Desgrandchamps,
Nathalie Rouas-Freiss and Edgardo D. Carosella

CEA, Research Division in Hematology and Immunology (SRHI), Saint-Louis Hospital, Paris, France

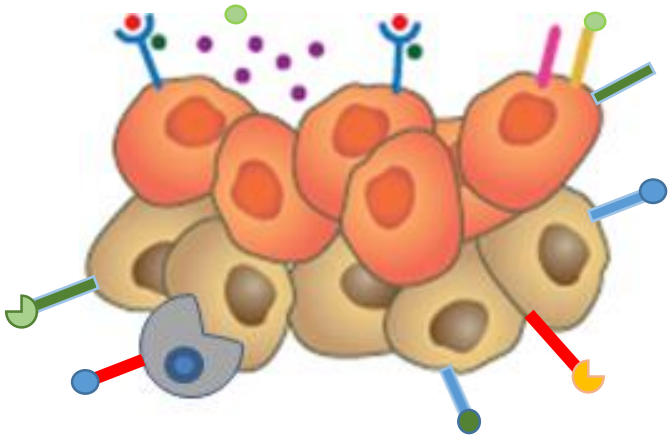
University of Paris, France

Service d'Anatomo-Pathologie, AP-HP, Hôpital Saint-Louis, Paris, France.

Service d'Urologie, AP-HP, Hôpital Saint-Louis, Paris, France.

The heterogeneity of cancer cells introduces significant challenges in designing effective therapeutic approaches.

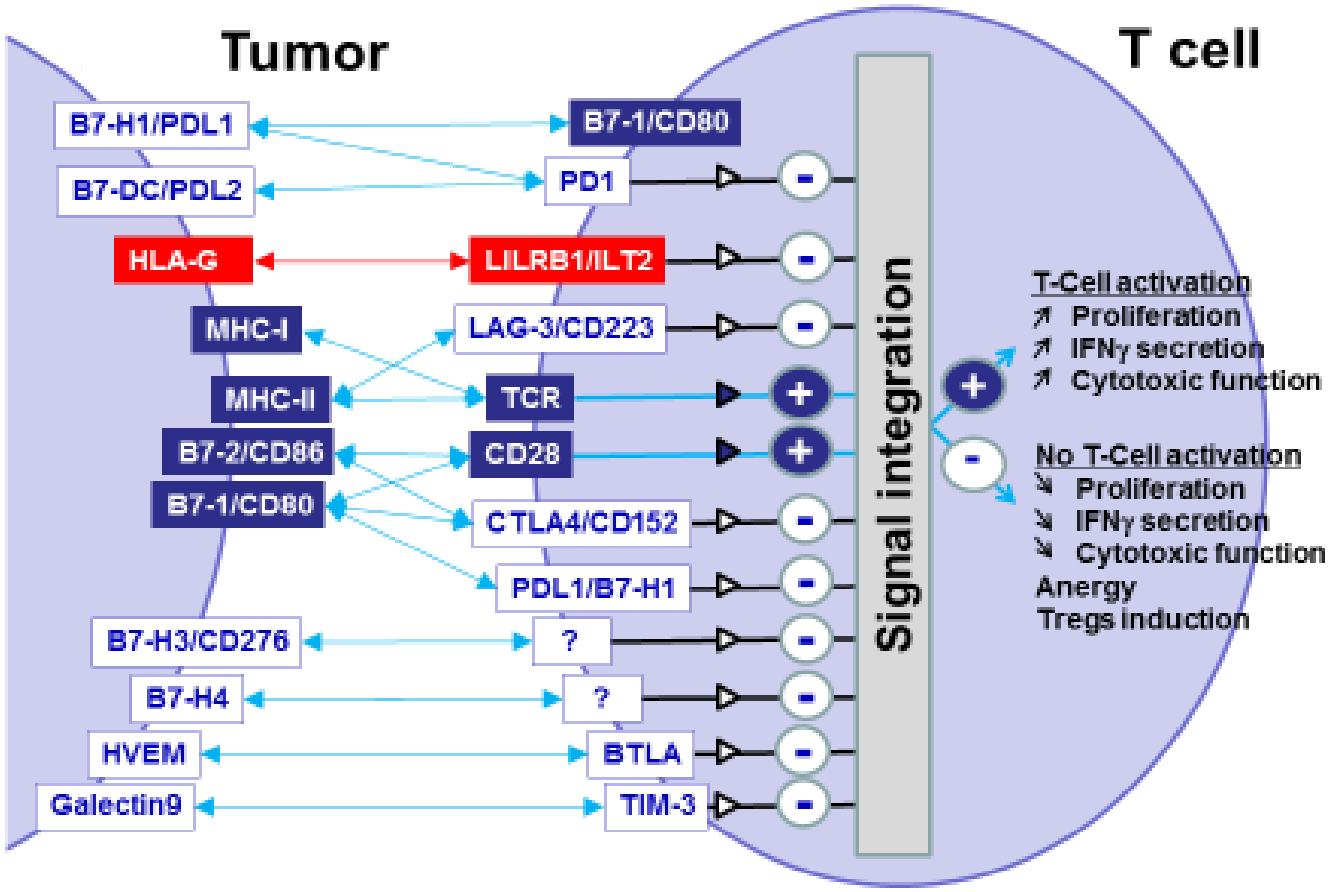
Model: Clear Cell Renal Cell Carcinome (ccRCC), a frequent malignant tumor of the adult kidney



immune-checkpoints
at the cell-surface

Cancer immunotherapy has positively revolutionized outcomes and basic concepts of oncological treatments.

Key immune-checkpoints at the interface between tumor cells and immune effectors

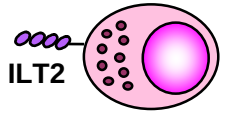
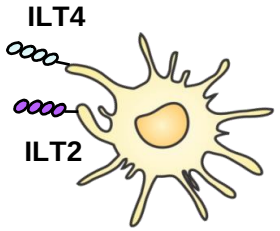
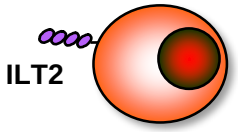
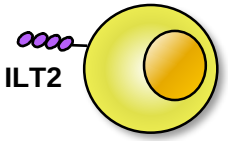
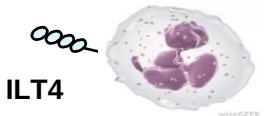


These immune-checkpoints (IC) have been broadly defined as cell-surface molecules that can transduce signals into effector cells to positively (stimulatory receptors) or negatively (inhibitory receptors) modulate signaling upon ligand binding. Tumor cells take advantage of these associations to escape destruction by the immune system.

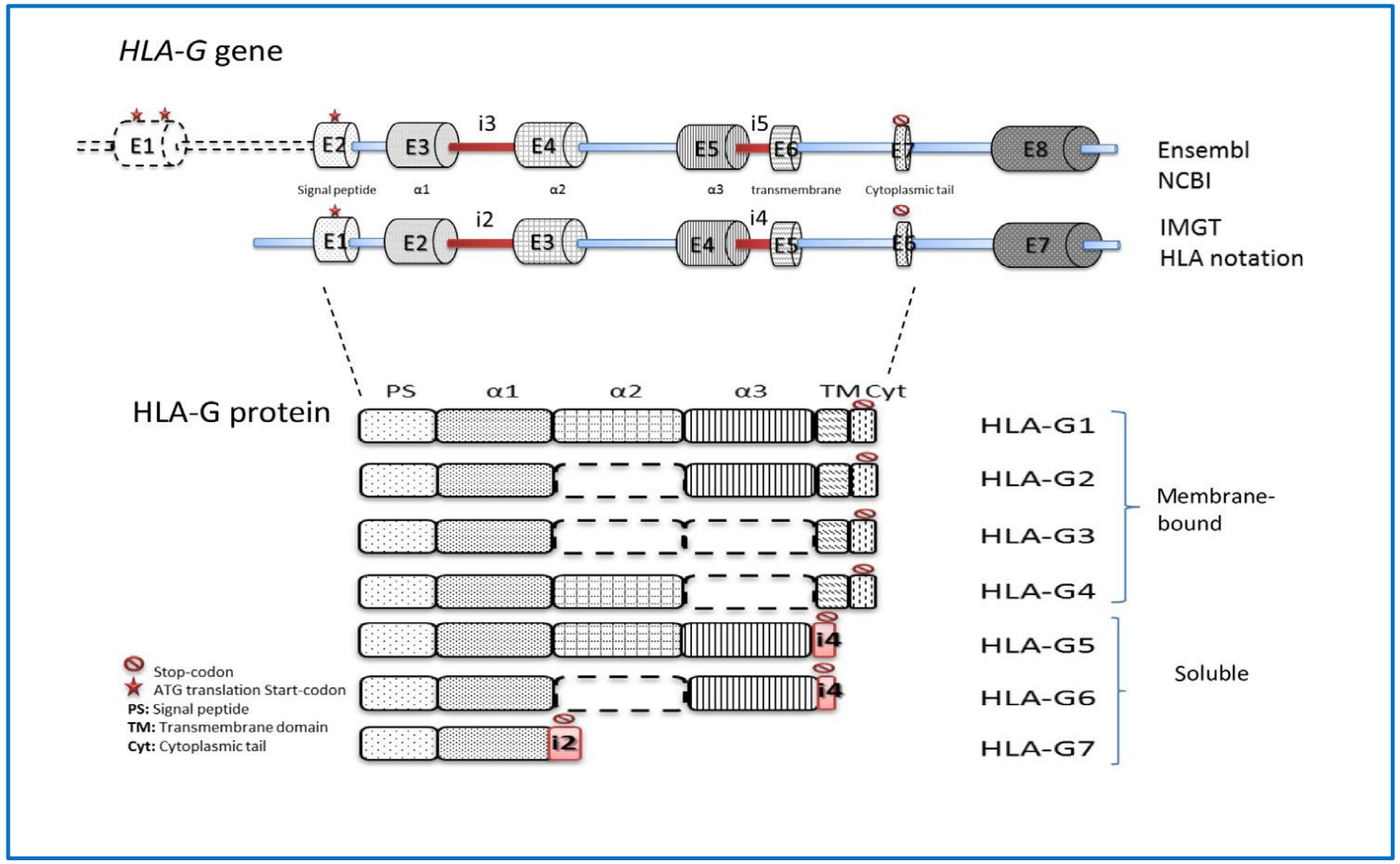
At present, antibody-based therapeutics targeting IC proved to improve prognosis and help establishing a more effective antitumor response.

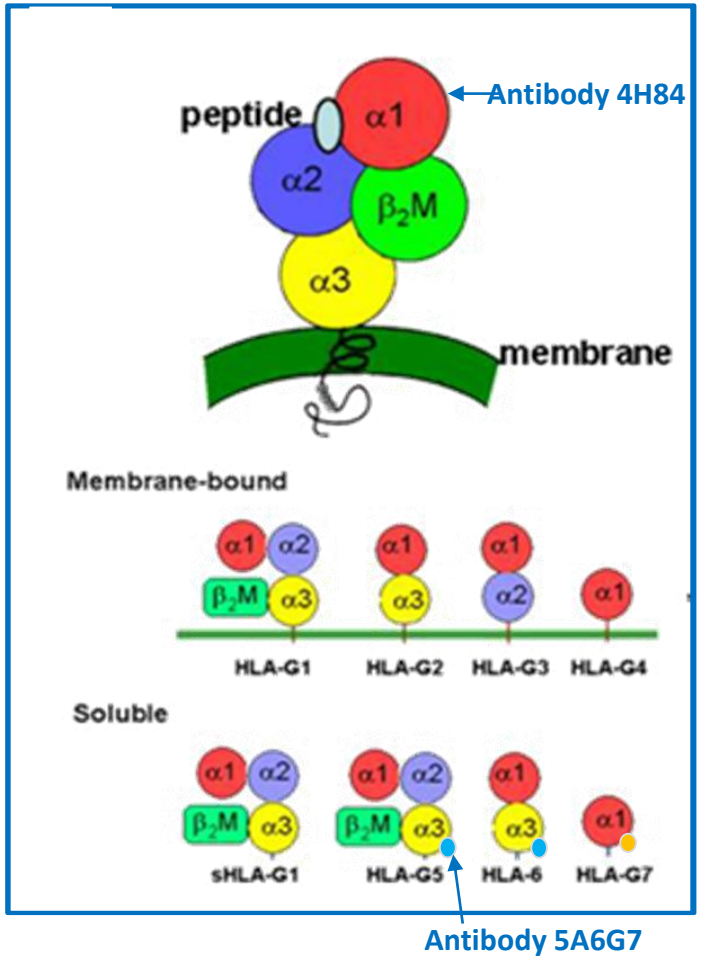
HLA-G: an Immune Checkpoint with Broad Immune-inhibitory Functions

- Involved in immune tolerance
- First described to play a crucial role in feto-maternal tolerance.
- Restricted expression in normal tissues
- Found in most of the tumors analyzed

	Immunological functions	
 NK cells	• Inhibition of **cytotoxicity** • Inhibition of **IFN-γ secretion** • Inhibition of **MICA/NKG2D activation** • Inhibition of **chemotaxis**	*Rouas-Freiss et al, 1997* *Favier et al, 2010* *Menier et al, 2002* *Morandi et al, 2011*
 Dendritic cells	• Induction of **tolerogenic DC** • **Inhibition of maturation** • Reduced MHC II **presentation** pathway • Decreased **Co-stimulatory molecules** • Induction of **anergic and suppressor T cells** • Inhibition of **NK cell activation**	*Ristich et al, 2005* *Gros et al, 2008*
 T cells	• Inhibition of **proliferation** • Inhibition of **cytolysis** • Induction of **Tregs** • Induction of **Th2-type cytokines** • Inhibition of **chemotaxis** • Inhibition of **γδ T cells**	*Bahri et al, 2006* *Le Gal et al, 1999* *LeMaoult et al, 2004* *Agaugue et al, 2011* *Morandi et al, 2010* *Lesport et al, 2011*
 B cells	• Inhibition of **proliferation, Ig secretion, and chemotaxis**	*Naji et al, 2014*
 Neutrophils	• Inhibition of **reactive oxygen species production and phagocytosis**	*Baudhuin et al, 2013*

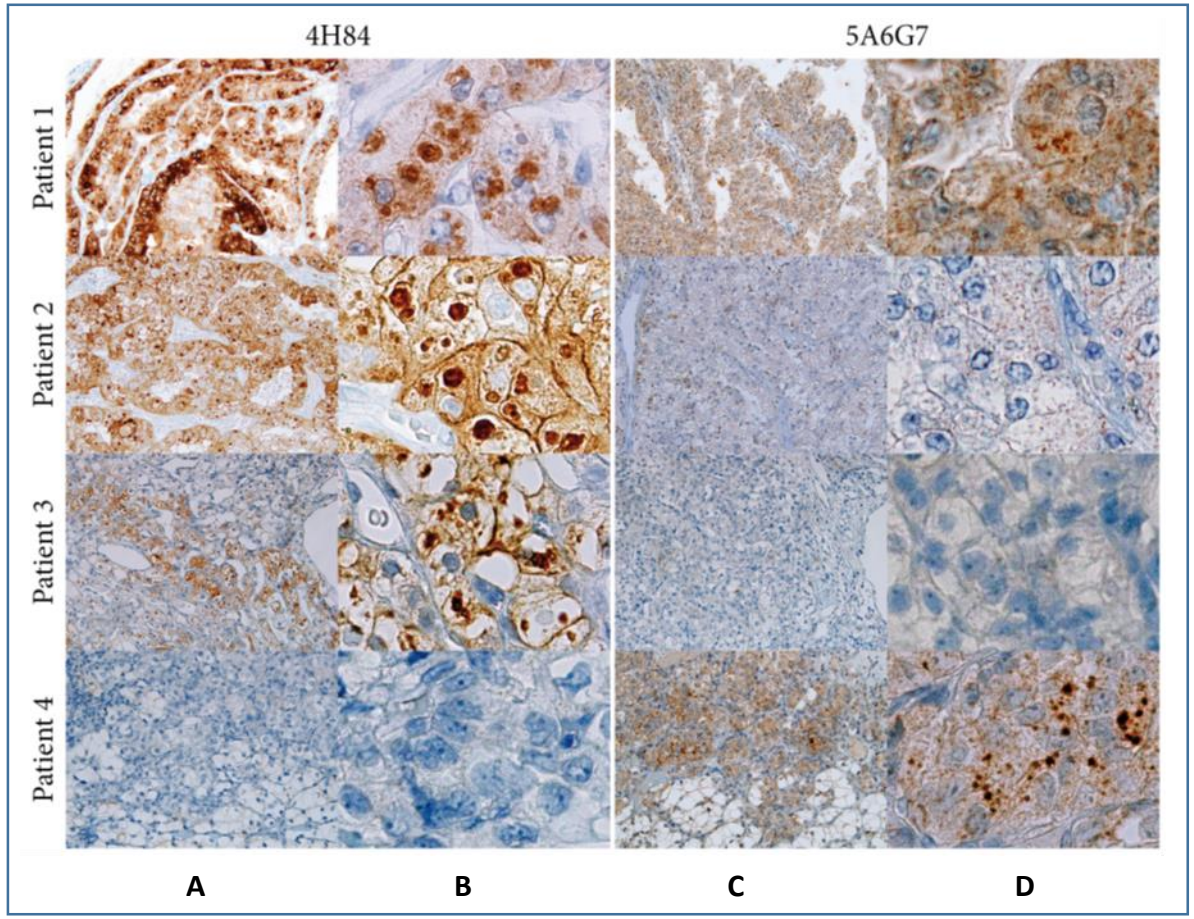
Structure of HLA-G and proteins





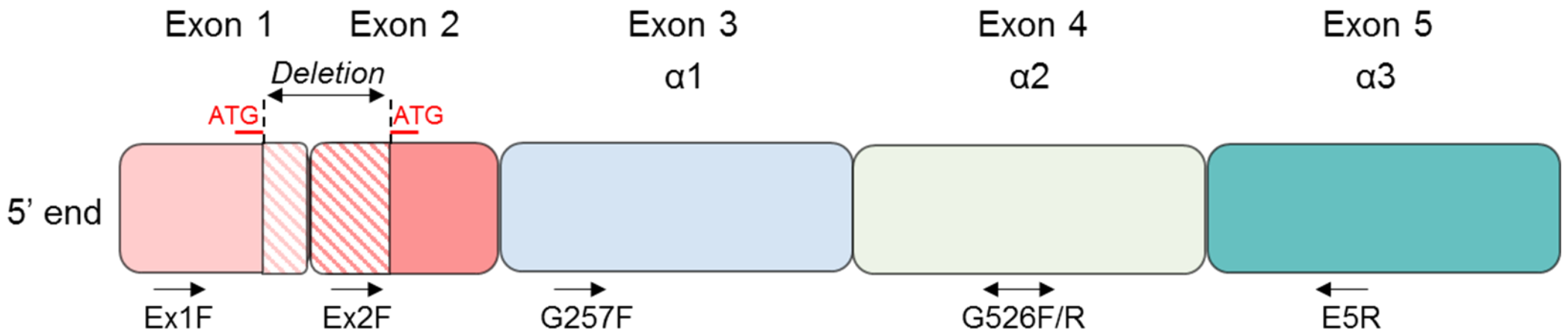
The primary transcript of HLA-G is alternatively spliced, producing at least seven mRNAs encoding four membrane-bound (HLA-G1 to HLA-G4) and three soluble (HLA-G5 to HLA-G7). These isoforms display one, two or three extracellular domains. The soluble proteins have retained intron sequences that include stop signals that prevent the translation of the transmembrane and intracytoplasmic domains. The expression of multiple isoforms in tumors, mainly produced by alternative splicing in a non-uniform distribution, might be in part, responsible for treatment failure.

Differential morphologic and HLA-G staining patterns of tumors of representative ccRCC patients



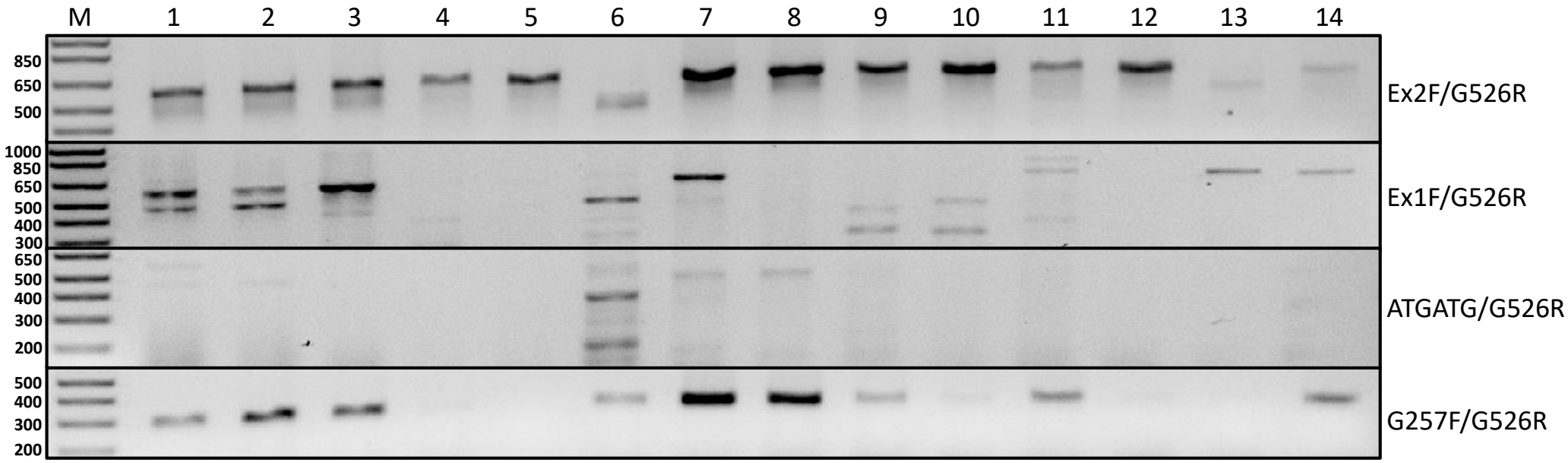
Immunohistochemistry analysis of HLA-G expression probed with mAbs 4H84 and 5A6G7. 4H84, which recognizes an epitope located into the a1 domain common to all seven reported HLA-G isoforms and the antibody 5A6G7 that only recognizes soluble HLA-G5 and HLA-G6 isoforms. This latter antibody targets the amino acids encoded by the retained intron 5 (previously known as intron 4 according to the IMGT/HLA nomenclature). A and C are overall primary ccRCC sections. B and D, specifically show hyaline globules.

Schematic representation of the PCR-based strategy to analyse HLA-G isoforms



Ensembl database nomenclature

Novel HLA-G isoforms are also present in trophoblasts

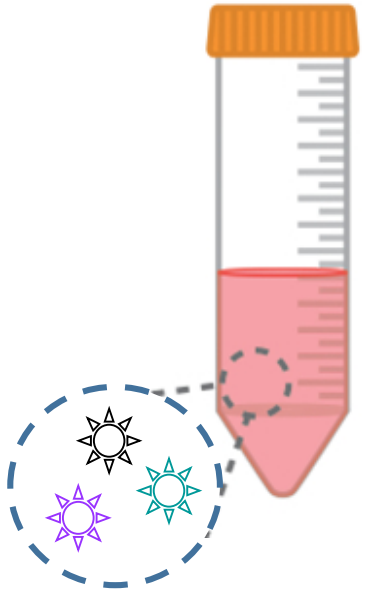


Trophoblastes	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
Ex2F/G526R	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ex1F/G526R	+	+	+	-	-	+	+	-	+	+	+	-	+	+
ATGATG/G526R	+	+	-	-	-	+	+	+	-	-	-	-	-	-
G257F/G526R	+	+	+	-	-	+	+	+	+	-	+	-	-	+

RT-PCR was performed on 14 trophoblasts using specific HLA-G primers

Cellular models expressing different HLA-G isoforms

Lentivirus encoding HLA-G1, HLA-G1L and HLA-GΔα1 (TAG)



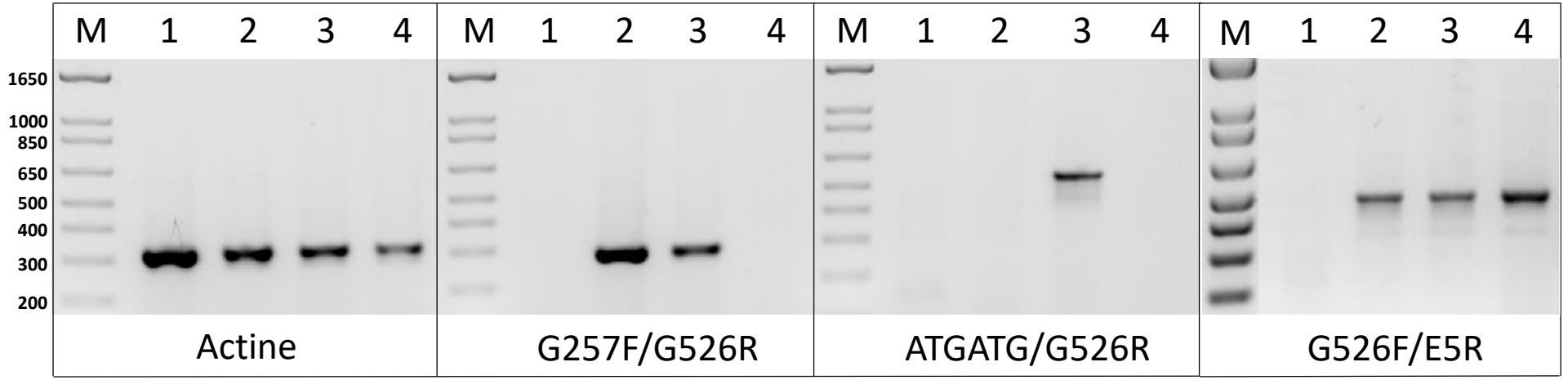
The different isoforms were expressed in two different cell lines to conduct structure-function studies.

RCC7 cell line which derives from a tumor of a patient with ccRCC

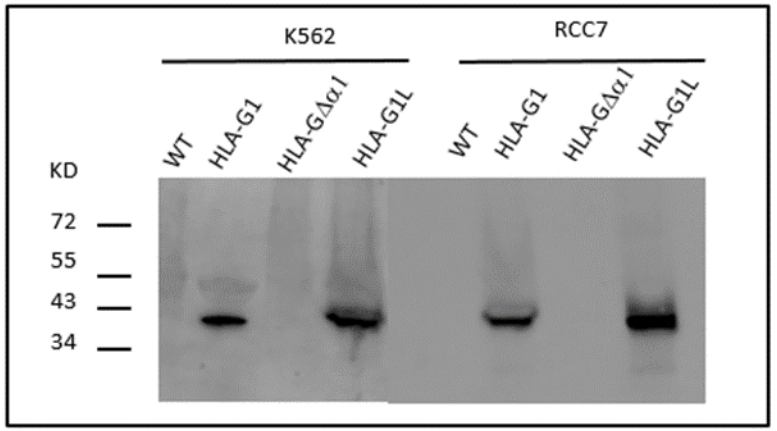
K562, erythroleukemic cell line commonly used as target cell for NK function studies

TAG: peptide DYKDDDDK added at the 3'end of HLA-GΔα1

HLA-G1, HLA-G1L and HLA-GαΔ1 are expressed at the mRNA and protein levels

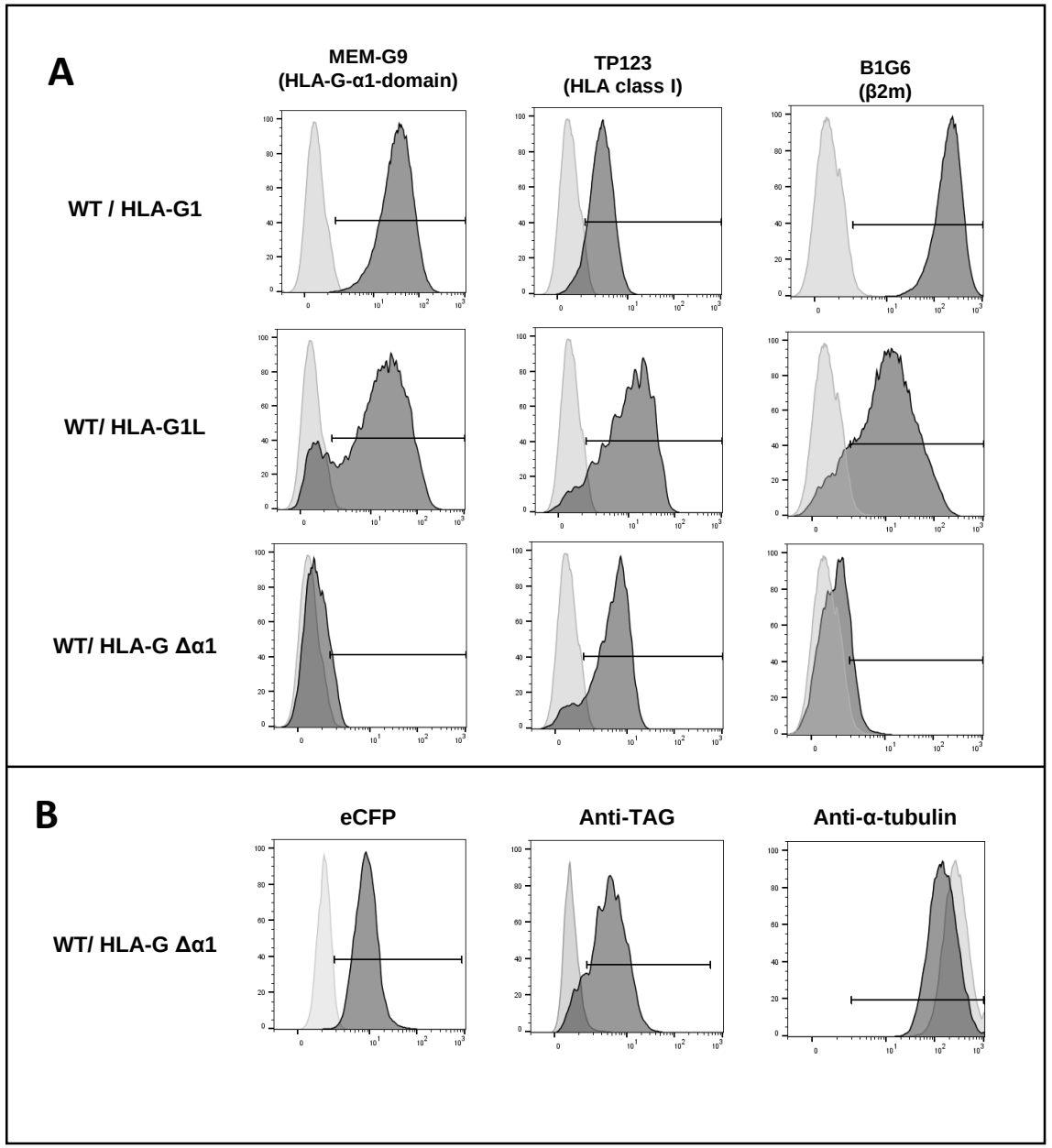


RT-PCR analysis using specific HLA-G primers. M= DNA Ladder (1 Kb plus) , 1= WT, 2= HLA-G1, 3= HLA-G1L, 4= HLA-GΔα1



Western blot experiments were first conducted using the 4H84 mAb, which specifically detects denatured HLA-G via the α 1 domain epitope. As expected we found that HLA-G1 and HLA-G1L transcripts were translated into a 39- to 40-kDa protein, in both K562 and RCC7 cell lines (Fig. 6). As predictable, the HLA-G Δα 1 protein missing the α 1 domain could not be detected in any cell line with 4H84 mAb

HLA-G1, HLA-G1L and HLA-GαΔ1 are addressed at the cell-surface.



Conclusions

✓ HLA-G, a recently identified immune checkpoint, promotes tumor survival.
Its blockade may provide therapeutic benefit against cancer.

✓ Marked heterogeneity of HLA-G isoforms distribution in tumors of ccRCC patients, including hyaline globules, may reflect functional differences.

✓ Currently available commercial anti-HLA-G antibodies are unable to recognize the novel HLA-G isoform lacking the α1 domain, underestimating the HLA-G expression in cancer lesions.

✓ The unreported HLA-G isoforms are also detected in placental trophoblasts.

✓ The structural conformation adopted by the novels HLA-G isoforms suggests:
- distinct interactions with ILT2 and ILT4
- new HLA-G functions

✓ The novel and extensive portrait of HLA-G isoforms should prove suitable for the effective tailoring of future clinical applications.