Sources of Antigenic Peptides for the MHC-I Pathway and its Significance for Viral Immune Evasion

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BACKGROUND

The peptides displayed on major histocompatibility class I (MHC-I) molecules are essential for the immune system to detect virus-infected or cancerous cells[1]. The peptide substrates can derive from a non-canonical mRNA translation event from intron sequences and 5´3´untranslated as well as from peptide splicing[2-4]. Our works have focused on the production of antigenic peptides by non-canonical mRNA translation for the development of new anti-viral therapeutic strategies.

Epstein-Barr virus EBNA1 Gly-ala repeat (GAr) has the ability to inhibit mRNA translation in *cis* thereby minimizing antigenic peptide production for the MHC-I pathway[6]. Recently, had came to light that this process is mediated through the interaction with a G-quadruplexes formed in GAr-encoding mRNA sequence and the host cell protein nucleolin. The G-quadruplex ligand PhenDC3 inhibits nucleolin binding to EBNA1 mRNA and reverses GAr-mediated inhibition of EBNA1 expression and antigen presentation[7]. Together, these characteristics make GAr an interesting tool for understanding non-canonical translation of antigenic peptide substrates.

CONCLUSION

GAr-mediated translation inhibition do not occur in the context of intron-bearing RNAs. In the majority of eukaryotic mRNAs, processing, nuclear export and translation is ensured by splice factors[6]. However, 95% of herpesvirus transcripts do not have introns[8], which is probably a strategy to avoid canonical mRNA processing and presentation of antigenic peptides substrates on MHC-I molecules.

Binding of nucleolin to the GAr mRNA is not necessary to retain it in the nuclear compartment and the ability of PhenDC3 to disrupt nucleolin binding and promote RNA export and protein synthesis are two independent effects. This result also raises the possibility that G - quadruplexes formed by GAr mRNA interact with other RNA-binding proteins than nucleolin. These factors might conduct GAr mRNA to a specific maturation pathway evolved by Epstein-Barr virus in order to restrain this message into the nucleus and prevent the production of antigenic peptides. In addition, the HCV IRES stimulates antigen presentation while suppressing canonical translation, indicating that full-length protein and antigenic peptide derive from distinguishable mechanisms.

Knowing which neoantigens are presented on MHC-I molecules is crucial to design new vaccines against virus-infected cells.

These results will lead to a better understanding of the underlying molecular mechanisms that control the synthesis of peptide substrates for the MHC-I pathway and open doors for new therapeutic approaches aimed at regulating the presentation on pathological conditions.

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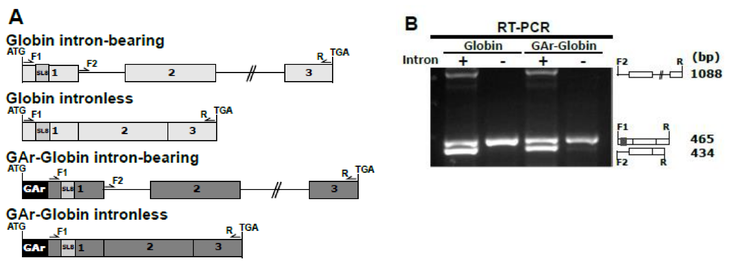
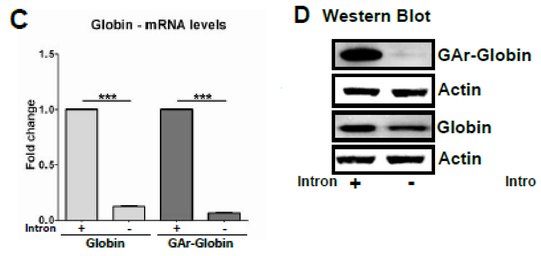
RESULTS

EBNA1 GAr does not suppress mRNA translation in the context of introns

In order to better understand the processes that control the production of alternative antigenic peptides and how GAr interferes in these events we started by testing the levels of protein synthesis in the context of introns. To do that we made four different constructs in which identical mRNAs expressing β-globin (globin) or chicken ovalbumine (OVA) where generated either via splicing from intron-bearing pre-mRNAs and from intronless cDNAs (Fig. A).

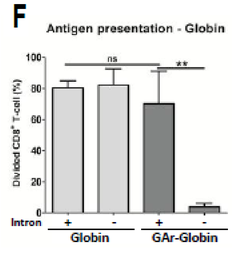


Performing a RT-PCR, we confirmed that the presence of the SL8 epitope or the GAr sequence did not affect the pre-mRNA splicing and that the mRNAs derived from spliced pre mRNA or from cDNA are the same (Fig. B).



Intron-bearing mRNAs gave rise to higher levels of mRNA, as compared to the corresponding intronless messages (Fig. C). When we compared the ration protein:RNA we observed that despite a higher level of mRNAs the presence of introns overcame the translation inhibitory effect of the GAr (Fig. D and E).

In order to test if a increase in protein synthesis in the context of introns would affect the capacity of GAr to control the production of antigenic peptides substrates, we incubate OT-1[1] CD8⁺ T cells against Ova-derived SL8 epitope with human H1299 cells expressing the murine MHC class I molecule (Kb) and indicated constructs and estimated antigenic peptide substrate production by determining OT-1 cell proliferation using FACS analysis.

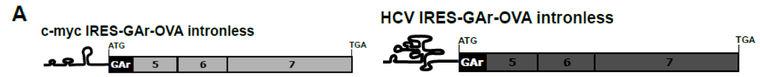


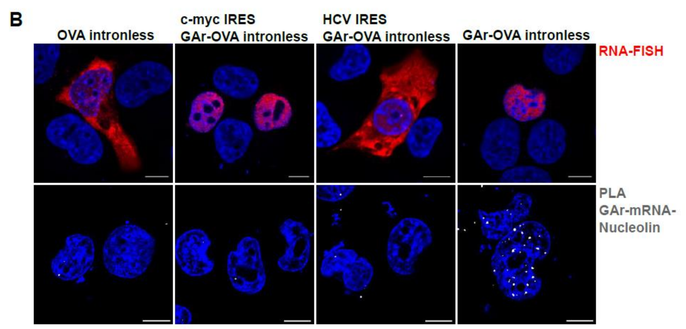
We observed that EBV GAr sequence in the context of intronless globin construct, suppressed antigen presentation and did not affect antigenic peptide production in the context of intron-bearing RNAs (Fig. F).

[1]OT-1 transgenic mice contain inserts for mouse Tcra-V2 and Tcrb-V5 genes that code the specific chains α and 8 of the T cell receptor that recognizes the SL8 epitope.

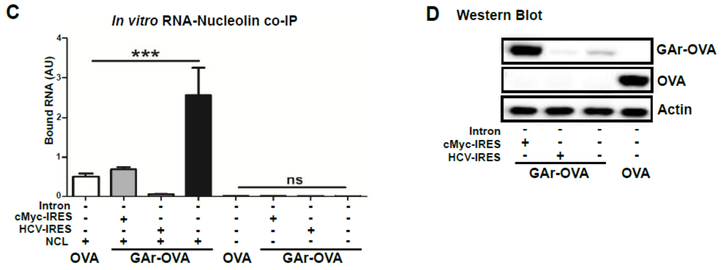
Altering the 5´UTR of GAr carrying mRNAs affects their subcellular localization, nucleolin interaction and the synthesis of antigenic peptide substrates

To addressed if mRNA nuclear retention is important in the ability of nucleolin to control GAr-mediated translation suppression we used the 5´untranslated region (UTR) of the c-myc and HCV sequences containing internal ribosome entry sites (IRESs) (Fig.A).

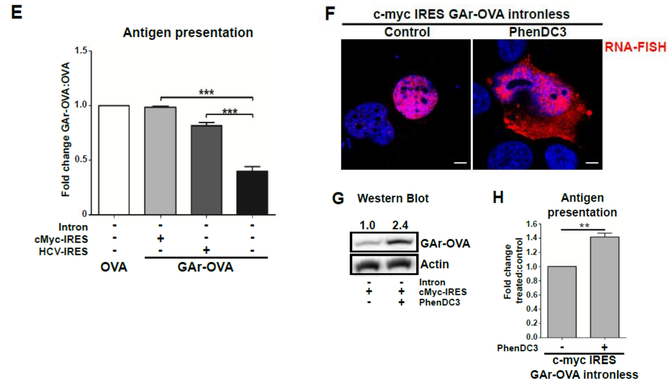




RNA FISH analysis showed c-myc IRES prevented interaction with nucleolin but had no effect on the localization of the intronless GAr-OVA mRNA. The HCV IRES also prevented the interaction with nucleolin but promoted the export of the GAr-OVA message to the cytoplasm (Fig. B)



In the *in vitro* RNA nucleolin co-IP using total RNA from transfected cells and recombinant nucleolin, we observed that the presence of the c-myc or the HCV-IRESs prevented the interaction with nucleolin (Fig. C). When we added HCV IRES in 5´UTR of intronless GAr-OVA mRNA we observed a further suppression of GAr-OVA protein expression (Fig. D)



Both IRESs overcome the ability of GAr to suppress antigen peptide production (Fig. E). After treating cells expressing c-myc IRES GAr-OVA intronless mRNA with PhenDC3 and evaluating the RNA localization by FISH, we saw that PhenDC3 promoted the nuclear release of this mRNA (Fig. F). PhenDC3 treatment also resulted in an increase of protein (Fig. G) and antigen presentation (Fig. H).