Title: Distinct mechanisms of loss of IFN-gamma mediated HLA class I inducibility in two melanoma cell lines.

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Author's response to reviews: see over
Dear Dr. Le,

February, 13 2007

We would like to thank you and the reviewers for reading our manuscript “Distinct mechanisms of loss of IFN-gamma mediated HLA class I inducibility in two melanoma cell lines” and making valuable comments and suggestions.

We would like to respond to the concerns of the reviewers.

Reviewer #1, Silvano Ferrini

Minor essential revisions:
1) Several typewriting errors throughout the manuscript have been corrected.

2) We have added the name of the cell line (ESTDAB-004) to the sentence “Examination of STAT-1….“ in the results paragraph of the abstract.

3) Legend to Fig.2 has been changed. The WB analysis was performed on total cell lysates without immunoprecipitation, and according to the protocol described in the materials and methods section.

4) Labels of the vertical axes on Fig.1 have been added.

5) We agree, the position of the pSTAT-1 band is a bit lower than in the positive control. This happens sometimes during electrophoresis when the gel is slightly overheated, making a “smiley face”-looking gel. We have replaced this picture with a better one obtained from another SDS-PAGE that had been run under the same conditions.

6) We have eliminated EGFR data from the manuscript according to the suggestion of another reviewer, and added the name of the control cell line: ESTDAB –056 (not –108). Corresponding changes are also made in the discussion.

7) The legend for Fig.5 has been modified. In the presence of anti-IRF-1 antibody the specific band in the Supershift assay disappears (lanes 7 and 8). The position of the shifted bands is not clear since they are just gone due to the blocking effect of the antibody.

Discretionary revisions:

1) In ESTDAB-159 cell line HLA class II expression was not upregulated after treatment with IFN-g further confirming the IRF-1 mediated defect in IFN-γ resistance mechanism in this cell line.
2) IRF-1 is induced by IFN-γ and ISGF3 is induced by IFN-α, and both of them bind the same ICS sequence motif on the HLA class I promoter. Therefore, we can speculate that the hypermethylation of the HLA class I promoter most likely is blocking one cytokine pathway more than another. Theoretically, we cannot completely exclude the possibility of partial methylation of HLA class I promoter, however, we cannot provide any experimental evidence supporting it. This is just a speculation. Thus, we discuss also other possibilities that have to be further investigated.

Reviewer #2, Catia Traversari

Major Revision:
1) The differences in the molecular mechanisms used by IFN-gamma and –alpha to control MHC-I expression are described in more detail in the introduction.

2) We agree, the position of the pSTAT-1 band is a bit lower than in the positive control. This sometimes happens during electrophoresis when the gel is slightly overheated, making a “smiley face“-looking gel. We have replaced this picture with a better one obtained from another SDS-PAGE that had been run under the same conditions.

3) We agree with your comment and have removed EGFR expression analysis from the manuscript.

4) This analysis is only qualitative, not quantitative. Therefore, we think that even is there was a small increase in the EGRF expression after IFN-gamma treatment, the sensitivity of the method would not have allowed seeing it.

5) The following paragraph is added: “While IFN-α is used in treatment of cancer with measurable efficacy (33), IFN-γ demonstrated limited success in cancer immunotherapy in humans. It might be explained by tumour-cell insensitivity to IFN-γ (34), or an inability to therapeutically recapitulate the natural periodicity of IFN-γ production. An understanding of the mechanisms by which tumors circumvent cytokine signaling would greatly aid prediction of the immune response in patients treated with IFNs”.

The corresponding references are included to the list:


34. Wong LH, Krauer KG, Hatzinisiriou I, Estcourt MJ, Hersey P, Tam ND, Edmondson S, Devenish RJ, Ralph SJ. Interferon-resistant human melanoma

Minor revisions:
1) Typos corrected
2) The name of the cell line -ESTDAB-004 – is included
3) IRF-1 spelled out
4) The typo is corrected
5) ISGF3 - INTERFERON-STIMULATED TRANSCRIPTION FACTOR 3 – added to the text
6) The reported absence of inducibility for MHC class II was referred to IFN-gamma and it has been added to the text
7) Figure 1A – Y-axis description added and the legend for this figure is now with more detail
8) This sentence is misleading and it has been deleted.
9) Fig.4 legend corrected.
10) Fig.6A - axis description added.
11) We changed the way the lines look in order to distinguish them better.
12) Cell line ESTDAB-056 was used as a control, text and figures have been corrected accordingly.

Discretionary revisions:

1) We agree, it would have been interesting to show the results of Jak2 phosphorylation in response to IFN-gamma and the basal level of SOCS1 in ESTDAB-159. However, one would expect that Jak2 is also phosphorilated in these conditions, since IFNg treatment did induce phosphorylation of STAT1 in this cell line.
2) Perhaps, it would be nice, however, the picture would have been too busy.

Looking forward to hearing form you soon.

Sincerely,

Natalia Aptsiauri, MD, PhD

Francisco Ruiz-Cabello, PhD