Author's response to reviews

Title: Heparan sulfate mediates trastuzumab effect in breast cancer cells

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Author's response to reviews: see over
To Dr. Calvin Roskelley  
Associate Editor  
BMC Cancer

Dear Professor,

We are forwarding the new version of the manuscript as well as the second revision answers of the article entitled: “Heparan sulfate mediates trastuzumab effect in breast cancer cells” (MS: 613864753103835).

Reviewer: Elda Tagliabue

Reviewer's report:
While the revised version of the manuscript was actually improved, in my opinion, the paper is still disjointed. It is, in fact, difficult to understand why transfection of HPSE-1 was considered a valid tool for understanding the role of HS in trastuzumab activity if HPSE-1 upmodulation also changes the expression levels of HER2. In my opinion, experiments showing correlation of trastuzumab activity and HS expression, as well as physical interaction between trastuzumab and HS and inhibition of trastuzumab activity by both anti-HS antibody and heparin, can represent by themselves convincing proofs of the role of HS in trastuzumab efficacy. On the contrary, it is still difficult to fully appreciate the part of the manuscript concerning trastuzumab capability to change GAG production.

Major Compulsory Revision
1. It should be better to firstly present data demonstrating that HER2 interacts with HS and that the block of HS decreases susceptibility of tumor cells to trastuzumab, and in sequence, those showing the capability of trastuzumab to affect expression of GAG as well as of HPSE-1 expression to modify HER2 levels.

Minor essential Revision.
2. English editing

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests

ANSWERS to the Major Compulsory Revision
As suggested by the reviewer the results were arranged in a different sequence. The authors would like to thank the reviewer and agree that this new version improved a lot the comprehension of the manuscript.

Briefly, the authors present the logic of the new sequence. Initially, it was demonstrated that there are responsive (SKBR3 and MCF7) and resistant (MCF7-HPSE1) breast cancer cells to trastuzumab (Figure 1), and that heparan sulfate is important to modulate the effect of this monoclonal antibody (Figures 2 and 3). These results led us to investigate heparan sulfate and also galactosaminoglycans synthesis in these different breast cancer lines and we have observed that responsive cell lines (SKBR3 and MCF7) presented a higher amount of heparan sulfate at cell surface...
compared to the MCF7-HPSE1, corroborating that heparan sulfate is important to trastuzumab response (Figure 4). The difference of HER2, HPSE1 and Syn-1 expression as well as HPSE1 activity was confirmed in the breast cancer lineages (Figure 5 and 6). Finally, it was investigated if trastuzumab could change the expression profile of HER2, HPSE1 and Syn-1. The data clearly demonstrated that trastuzumab decreased the expression of these molecules in responsive breast cancer cells (MCF7 and SKBR3), whilst an opposite result was obtained in the resistant lineage (MCF7-HPSE1), Figures 7 and 8.

Concerning the second point raised by the reviewer “why transfection of HPSE-1 was considered a valid tool for understanding the role of HS in trastuzumab activity if HPSE-1 upmodulation also changes the expression levels of HER2”, we present the following explanation.

ANSWER

The authors agree with the reviewer considering that HPSE1 transfected cell line (MCF7-HPSE) downmodulated HER2 expression and this effect could contribute to trastuzumab resistance in this cell line. However, it is important to point out that HER2 expression was increased after trastuzumab treatment in the transfected cells. Despite HER2 upmodulation MCF7-HPSE1 remains resistant to trastuzumab. If HER2 were the main determinant for trastuzumab effect, MCF7-HPSE1 would become responsive after the treatment. Moreover, responsive cells (SKBR3 and MCF7) treated with trastuzumab decrease HER2 expression but maintained the response to this antibody.

Furthermore, it was also demonstrated that HPSE1 transfection in MCF7 cells, promoted a reduction in the cell surface heparan sulfate compared to the responsive cell lines (SKBR3 and MCF7). These results, combined with the experiments that determined the interaction of heparan sulfate and trastuzumab, proved that HER2 expression is not the only determinant to trastuzumab effect in breast cancer cells and also that cell surface heparan sulfate modulates this effect.

It was added in the new version of the manuscript the following phrase in the line 513: “Despite HER2 upmodulation after trastuzumab treatment, MCF7-HPSE1 remains resistant. If HER2 were the main determinant for trastuzumab effect, MCF7-HPSE1 would become responsive after the treatment.”

ANSWERS to the Minor Essential Revision

As suggested by the reviewer the new english revision of the manuscript was performed by the native professional translator Glenn C. Johnston.

Yours sincerely,

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