Reviewer's report

Title: A-770041 reverses paclitaxel and doxorubicin resistance in osteosarcoma cells

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Reviewer: Silvio Naviglio

Reviewer's report:

The manuscript entitled “A-770041 reverses paclitaxel and doxorubicin resistance in osteosarcoma cells” by Zhenfeng Duan et al, reports on the identification of the Src family kinase inhibitor A-770041, acting as one of the most effective multidrug resistance (MDR) reversing agents when combined with doxorubicin or paclitaxel in human osteosarcoma MDR cell lines.

The paper is well written, pretty straightforward and is containing new information. In addition the manuscript is of appropriate length and its design is clear.

The experimental approach is well performed and results support enough the conclusions of the Authors.

Nevertheless, this reviewer feels some changes and additional data would greatly increase the reliability of this study and asks some “easy to perform” amendments which will improve the overall quality of the paper.

Finally, the reviewer considers the manuscript worth of publication, once the questions apart mentioned will be addressed.

Major Compulsory Revisions

As far as “Effects on drug sensitivities from inhibiting Src expression by shRNA” concerned (Fig 2), Authors claim that the Src protein expression in U-2OSMR or KHOSR2 cells was down-regulated using lentiviral Src kinase shRNA. However, Src protein levels are not shown.

In addition, results would be more convincing and conclusions by the Authors would be highly supported if they would have provided data that include also the evaluation of drug-induced cytotoxicity after transduction with control shRNA.

As far as “Synergistic effect of A-770041 with paclitaxel or doxorubicin in drug resistant cell line” concerned (Fig 3), in the relative legend is indicated that “The results are shown as the mean value of triplicate samples and are representative of 3 independent experiments”. However, statistical data (mean value, standard deviation…) are not shown in such figure.

As far as “Effect of A-770041 on the expression and activation of Src, Lck” concerned (Fig. 4), it is claimed that “A-770041 inhibits Src and Lck expression in drug-resistant osteosarcoma cells in a dose-dependent manner” and that “Western blot analysis revealed that A-770041 inhibits both Src and Lck activation and expression in osteosarcoma MDR cells, but has less or no effect
on other kinases such as pAKT, pmTOR or CDK11”.

It’s not completely true. I mean, in Fig. 4 it is shown only p-Src and p-Lck protein levels and not also total Src and Lck protein levels (even in “Methods Section, anti-Src and anti-Lck antibodies are not mentioned). In my opinion, statistical and densitometric analysis of pSrc/Src and pLck/Lck is critically needed to support the above claim, and it should be provided.

In addition, pAKT, pmTOR protein levels (at least in U-2OSMR cells) also appear down regulated in response to A-770041 treatment.

As far as “A-770041 enhances apoptosis induced by doxorubicin in drug resistant osteosarcoma cells” concerned (Fig. 5), it is claimed that “PARP cleavage was detected after the treatment of U-2OSMR or KHOSR2 cells with A-770041 in combination with doxorubicin (Figure 5B)”.

In my opinion, the data reported in such figure are not so much obvious and PARP cleavage is not clearly evident.

In addition, my feeling is that results would be more convincing and conclusions by the Authors would be highly supported if they would have provided data that include the monitoring of caspases activation also by Western blot and/or Flow cytometric analysis of apoptosis.

Discretionary Revisions

Bibliography is not completely adequate and updated. For instance, recent evidence on sensitization to doxorubicin in osteosarcoma cells by different agents is not cited.

Some misreadings throughout the text

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests