論文審査の要旨及び担当者

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試問日：2021年 7月 2日

（論文審査の要旨）

論文題名：A transposon screen identifies enhancement of NF-κB pathway as a mechanism of resistance to eribulin
（トランスポゾンで同定されたNF- κB経路活性化による乳癌のエリブリン耐性メカニズム）

Summary of the work

Background: Eribulin is a microtubule inhibitor that is frequently used in clinical practice for the treatment of metastatic breast cancer. However, the mechanism of drug resistance to eribulin remains unclear.

Aim of the work: In order to validate this mechanism and identify predictors of efficacy, we investigated the association of eribulin sensitivity with TAB2-NFκB which is identified via transposon screen.

Results: Activation of the NFκB pathway associated with TAB2 expression induces drug resistance of eribulin *in vitro*. Furthermore, for triple-negative breast cancer, the combination of eribulin with NFκB inhibitors showed a higher tumor growth inhibition effect *in vivo*.

Conclusion: TAB2-NFκB axis correlated with response of breast cancer to eribulin treatment, and clinical investigation of whether the effect of eribulin can be predicted by TAB2-NFκB will lead to appropriate drug selection.

Questions & Answers

The first question was why I chose TAB2 among the candidate genes. I answered this question as follows. Since several NFκB-related genes were identified as candidate genes during the screening process, we decided to focus on the relationship between the NFκB pathway and eribulin in this study. TAB2 is a much well-known stimulator of the NFκB pathway, and the TAB2-NFκB axis was also observed in breast cancer.

The next question was what the relation of TAB2 with breast cancer is. TAB2 can reactive repressed estrogen receptor signaling pathway, and associates with tamoxifen resistance.

I was also asked how to recognize the activity of NFκB and its role in breast cancer development. To this question, I replied that the most common active form of NFκB is phosphorylated p50-p65, and its activation can be confirmed by nuclear transfer of NFκB protein by nuclear localization sequences. NFkB pathway can induce breast carcinogenesis, metastasis, and chemotherapy resistance.

Another question was why the combination of NFκB inhibitors and eribulin is more effective *in vivo* than *in vitro*. It is assumed that the difference between *in vivo* and *in vitro* was due to the tumor microenvironment, as NFκB inhibitors could induce suppression of tumor-adjacent stromal cells *in vivo*, enabling improved tumor control.

In response to the question of if there were any examples of NFκB inhibitors being used on patients in clinical practice, it was answered that bortezomib is already approved for multiple myeloma treatment.

In response to the question of why MCF7 was chosen as the cell line to be used for transposon screening, previous studies have shown that stable drug screening can be performed with MCF7. We also selected this cell line because the target of eribulin in clinical practice is luminal breast cancer.

To the question as to the transposon insertion site of the TAB2 gene, it was answered that the insertion site is located in the intron.

The question was asked if the TAB2 expression in the screened MCF7 clones had been verified. Although this could not be verified in the clones used for screening, the correlation between TAB2 expression and sensitivity to eribulin was observed in several cell lines, suggesting that the results are highly reproducible.

Finally, I was asked whether software or other methods were used to verify the concentration of the bands on the Western blot. No software was used, but the same experiment was repeated three times, and the results were all similar, so the probability was considered high.

Although many points need to be clarified through further research, the experiments proved that the use of NFκB inhibitors increased the effect of eribulin. This study was judged to be significant because it was thought to have provided a baseline for clinical application in the future.