論 文 要 旨

ATP7B expression confers multidrug resistance through drug sequestration.

（ ATP7B の発現は薬剤の小胞への隔離を介して
抗がん剤多剤耐性をもたらす ）

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【序論および目的】back ground and purpose

ATP7A and ATP7B are copper transporters existing in the Golgi membrane and share 67% amino acid identity. They have six metal binding sites (MBS) in the N-terminal cytoplasmic region and 8 membrane spanning segments. We previously found that ATP7A confers multidrug resistance (MDR) of cancer cells and high expression of ATP7B confers resistance against cisplatin. MDR is caused by expression of some ABC transporters, modification of DNA repair systems, apoptosis defects and several more mechanisms. However there are no established therapies to overcome MDR in practice so far.

We evaluated drug sensitivities of ATP7B expressing cells to make clear that ATP7B also confers MDR and examined the mechanism of ATP7B mediated drug resistance with mutant ATP7B expressing cells and imaging of doxorubicin localization.

【材料および方法】material and methods

We used epidermoid carcinoma KB-3-1 cells as parental cells, wild type (wt) human ATP7B expressingt KB/WD cells and empty vector transfectant KB/EV cells. To investigate the roles of the MBSs, we used three mutant ATP7B (Cu0, Cu6 and M6C/S) expressing cells. Cu0 has no MBSs, Cu6 has only the sixth MBS and M6C/S (in the sixth MBS cysteines are replaced to serines). The drug sensitivities to cisplatin, doxorubicin, SN38, etoposide and paclitaxel of the cells are evaluated with MTT assay. We observed the ATP7B localization using EGFP tagged ATP7B expressing cells by a confocal laser microscope. The effects of NH_4Cl and tamoxifen on the drug sensitivities of KB/WD and KB/EV cells were examined using Luminescent Cell Viability Assay Kit.

【結　果】results

KB/WD cells are significantly resistant to doxorubicin (17.25fold), SN-38 (38.14 fold), etoposide (25.84 fold), and paclitaxel (2.00 fold) as well as cisplatin (12.03fold) in comparison with

KB/EVcells. Cu6 expressing cells are less but significant resistant to anticancer agents. However Cu0 and M6C/s expressing cells are sensitive to anticancer agents. Doxorubicin is sequestrated into the late endosome compartment from the nuclei in wt ATP7B and Cu6 expressing cells independently from copper at 4 hours after doxorubicin exposure. Wt and Cu6 ATP7B relocalized to the late endosome in the presence of doxorubicin. However doxorubicin localized to the nuclei in Cu0 and M6C/S expressing cells. NH_4Cl and tamoxifen suppressed late endosomal sequestration of doxorubicin, thereby attenuating drug resistance of KB/WD cells.

【結論及び考察】discussion and conclusion

ATP7B confers MDR in cancer cells by facilitating nuclear efflux and following late endosome drug sequestration. The sixth MBS of ATP7B is critical for doxorubicin resistance and relocalization of ATP7B. The sequestration of anticancer agents depends on the acidity of the vesicles partly.

It has been reported that ATP7B expression elevate in several human malignancies, including ovarian, gastric, and breast cancers when compared with non-cancer tissues and its high expression is a poor prognostic marker in ovarian and oral squamous cell cancers treated with cisplatin-base chemotherapy. According to our analysis and previous reports, ATP7B is expected to be important position in MDR of human cancers clinically as well as cisplatin resistance.

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