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ATP-gated P2X7 cation channel synergistically potentiates the cytotoxic effect of doxorubicin in lymphoma model (*in vitro*)

Background

Chemotherapy can activate the immune system by eliciting immunogenic cell death (ICD) of cancer cells mediated by the release of danger signals, including ATP. The purinergic P2X7 receptor is an ATP-gated cation channel expressed by hematopoietic as well as by many tumor cells (Scheuplein *et al.* 2009). Basal activation of P2X7 is beneficial for tumor growth, but strong stimulation of P2X7 can kill tumor cells (Roger *et al.*, 2015). Little is known about the role of P2X7 in the context of chemotherapy. We therefore studied the effects of P2X7 stimulation on Doxorubicin (DOX) toxicity in lymphoma cell lines

Objective

To evaluate P2X7 as a possible therapeutic target to improve cancer therapy.

Materials & Methods

Cell lines: Yac-1 and A20 (mouse T- and B cell lymphoma lines, respectively).

Drugs: ATP, doxorubicin (DOX), and Bortezomib (BTZ).

Cell death and pore formation were monitored by DAPI uptake and the acid phosphatase cell death assay. Pre-apoptotic phosphatidyl-serine exposure and translocation of Calreticulin to the cell surface were monitored by flow cytometry.

Summary

- ATP (200μM) increased the sensitivity of the cells to the toxic effects of chemotherapeutic drugs i.e. DOX and BTZ (Fig. 2 & 3). This effect was dependent on P2X7, since it was blocked by pre-incubating the cells with the P2X7-inhibitory nanobody 13A7.
- Co-administration of ATP with DOX significantly increased DOX uptake within one hour of treatment (Fig. 2C), although no cytotoxic effects could be observed at this time point (not shown). This is likely the result of P2X7 mediated membrane permeabilization.
- In P2X7-transduced A20 mouse lymphoma cells, ATP increased the surface translocation of Calreticulin and phosphatidyl-serine, a sign of ER stress and apoptosis. This effect was reversed by the P2X7 antagonist nanobody 13A7 (Fig. 4A & B)

Results

Fig. 2) Gating of P2X7 increases the uptake of DOX and synergistically enhances its cytotoxicity in Yac-1 cells

Yac-1 cells were incubated overnight with the indicated concentrations of DOX and ATP, and stained with DAPI to indicate cell death. In B, pre-incubation with the P2X7-inhibitory nanobody 13A7 shows that the effects of ATP are P2X7-dependent. In C, presence of ATP significantly increases the uptake of Dox.

Fig. 3) P2X7 activation augments BTZ induced cytotoxicity

A20 cells were incubated overnight with the indicated concentrations of BTZ and ATP, and stained with DAPI to indicate cell death. In B, pre-incubation with the P2X7-inhibitory nanobody 13A7 shows that the effects of ATP are P2X7-dependent.

Fig. 4) P2X7 activation enhances ER stress

A20 cells were incubated for 2 hours with 200 μM ATP and stained for extracellular Calreticulin (Figure A). In B, cells were treated with indicated concentration of DOX and ATP for 6 hours and stained with Annexin v to observe phosphatidyl-serine surface exposure.

Conclusion

- P2X7 synergizes the cytotoxic effects of chemotherapeutic agents to stimulate the immunogenic cell death pathway (mainly P2X7 k variant). The possible mechanisms for synergistic cytotoxicity are:
 - Increased cell permeabilization may enhance the chemotherapeutic drugs uptake
 - High intracellular Ca^{2+} and reactive oxygen species induced ER stress, may also results in phosphorylation of eIF2α and translocation of Calreticulin (CRT) on cell surface
- P2X7_k expression is beneficial for host by potentiating antitumor immune response.

References:

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- Roger S, Jelassi B, Coullin I, Pelegrin P, Besson P, Jiang LH (2015). Understanding the roles of the P2X7 receptor in solid tumour progression and therapeutic perspectives. Biochim. Biophys. Acta - Biomembr. 1848, 2584–2602. doi:10.1016/j.bbmem.2014.10.029