Repositioning disulfiram as a radio-sensitizer against atypical teratoid/rhabdoid tumors (AT/RT)

Seung-Ki Kim, Seung Ah Choi, Young Eun Lee, Kyu-Chang Wang, Ji Hoon Phi, Ji Yeoun Lee, Sangjoon Chong

Division of Pediatric Neurosurgery
Seoul National University Children’s Hospital
Seoul, Korea
M/11 mo

8/25/2010

STR (90%), 8/30/2010

8/25/2010

Tracheostomy (9/10/2010)
Gastrostomy (9/14/2010)

9/21/2010

HDC & PBSCT (9/21/2010)

11/22/2010

VP shunt, 4/7/2011
RT, 4/8/2011

3/26/2011

5/17/2011

Expired at 10 mo
Brain tumor initiating cell

- We isolated and characterized cells with high ALDH1 activity from various primary brain tumors by Aldefluor staining and FACS analysis (0.3 to 29% as ALDH1+, ALDH1+ in 23.5% of AT/RT).
- ALDH1+ cells have characteristics that are expected of BTICs:
  - grow as neurospheres
  - express NSC markers
  - are able to produce neurons as well as glial cells
  - high proliferative potential
  - harbor genes that are needed for the generation of iPS
  - high tumorigenic potential of ALDH1+ cells in vivo
- Targeted knockdown of ALDH1 by shRNA interference in BTICs potently disturbed their self-renewing ability

- We demonstrated the therapeutic effects of disulfiram against BTICs from AT/RT.
- The effects might be attributed to the modulation of stemness and metabolism.
- Considering the lack of an effective chemotherapy regimen for AT/RT, disulfiram may be an possible treatment option against AT/RT.
Atypical teratoid/rhabdoid tumors (AT/RT)
One of the most common aggressive brain tumors in infants

Brain tumor initiating cells (BTICs)
A type of cancer stem cell (CSC), retaining stem cell-like properties, such as self-renewal, pluripotent differentiation and the expression of stem cell markers

Aldehyde dehydrogenase (ALDH)
A polymorphic enzyme responsible for the oxidation of aldehydes to carboxylic acids, and is now used as one of the CSC markers for many cancers

Disulfiram (DSF)
Alcoholism treatment drug that inhibits the conversion of acetaldehyde to acetic acid to irreversibly inhibit ALDH

Repositioning (redirection, repurposing, repurfiling)
The process of finding new uses outside the scope of the original medical indication for existing drugs
Aim

- To evaluate anti-cancer therapeutic effect of DSF and RT combination against AT/RT \textit{in vivo}.
- To propose the possible mechanism of the radio-sensitizing activity of DSF on AT/RT cells.
Materials and Methods

- AT/RT cells
  - SNU.AT/RT-5 (9 mo old boy) & SNU.AT/RT-6 (13 mo old boy)
  - BT-12 & BT-16 (Dr. Peter Houghton)

- Cell viability assay to obtain IC$_{10}$ & clonogenic assay

- Western blot for DNA double strand break markers ($\gamma$-H2AX, p-DNA-PKcs, p-ATM), apoptosis marker (cleaved PARP, Survivin, Bcl2), autophagy maker (LC3B), NF$\kappa$B and p21

- Autophagy assay

- Immunofluorescence of $\gamma$-H2AX foci

- Animal experiments
  - Short-term therapeutic efficacy: tumor volume & immunofluorescence analysis
  - Long-term therapeutic efficacy: *In vivo* live imaging & survival analysis
In vitro radio-sensitizing effects of DSF on AT/RT cells

Sensitizer enhancement ratio: 1.2~1.6
Radio-sensitizing mechanisms of DSF

SNU_AT/RT-5

Control | DSF | RT | DSF+RT

SNU_AT/RT-6

Control | DSF | RT | DSF+RT

BT-16

Control | DSF | RT | DSF+RT

BT-12

Control | DSF | RT | DSF+RT

Stem cell
ALDH
p-DNA-PKcs
p-ATM
γ-H2AX
NF-κB
Cleaved caspase-3
Survivin
Bcl2
LC3B
p21
β-actin

DNA double-strand break

Apoptosis

Autophagy

Cell cycle arrest

55 KDa
460 KDa
100 KDa
17 KDa
69 KDa
17 KDa
16 KDa
25 KDa
16 KDa
21 KDa
42 KDa
Effects of DSF on irradiated AT/RT cells

DNA double-strand break

Apoptosis

Autophagy
Treatment protocol
In vivo radio-sensitizing effects of DSF
Long term treatment effects of DSF and RT combination therapy

![Diagram showing treatment cycles and survival rates](image)

- **A**: Diagram illustrating the treatment cycles with DSF, RT, and saline.
- **B**: Graph showing bioluminescence image units over days after injection of BT-16 cells.
- **C**: Survival curve showing percent survival over days after injection of BT-16 cells.

129 days
Conclusions

- DSF and RT combination therapy has additive therapeutic effects on AT/RT by potentiating programmed cell death including apoptosis and autophagy.
- Concomitant chemo-radiotherapy using DSF might be incorporated into conventional regimens for young AT/RT patients.