Cells 2025 Conference

The 3rd International Online Conference on Cells

25–27 March 2025 | Online

MDPI

Research on the identification of effective radiosensitization target genes in cancer cells

ZONGXIANG ZHANG[1], Barkha Saraswat[1], Zhongming Gao[1], Ankitha Vadi Velu[1], Ying Tong[1], Mitsuko Masutani[1]

1.Dept. Molecular & Genomic Biomed., CBMM, GSBS, Nagasaki Univ.

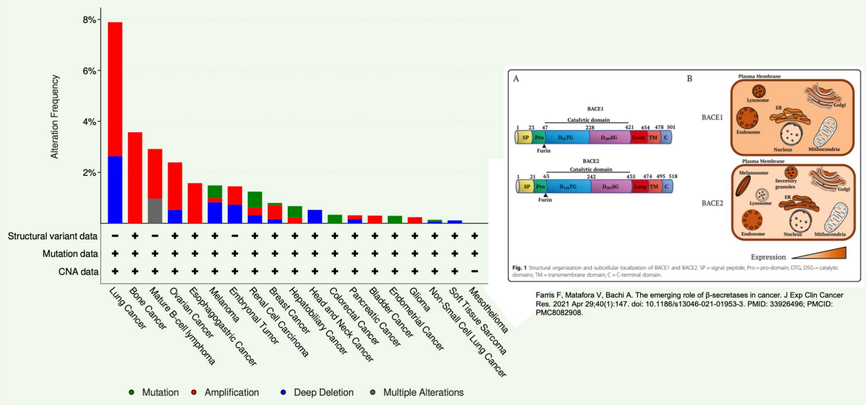
INTRODUCTION & AIM

The development of radiation sensitization methods for cancer cells with low toxicity to normal cells is considered to contribute to improving cancer radiotherapy. To date, a comprehensive approach using an shRNA library has been employed to identify candidate genes for γ-ray sensitization in cancer cells, with a focus on the *BACE1* (β-site of amyloid precursor protein (APP)-cleaving enzyme 1) gene. The *BACE1* gene mutation has been observed in lung cancer, bone tumors, ovarian cancer, and esophageal cancer. Meanwhile, the effect of inhibiting *BACE2* on radiosensitivity, which functions similarly to *BACE1* and is upregulated in cancer cells, has not been investigated.

It has previously been reported that the inhibition of *BACE1* leads to an increase in γ-H2AX foci, a marker of DNA strand breaks, suggesting its involvement in the early response to DNA damage. To investigate this possibility, *BACE1* was knocked down using siRNA in cancer cell lines, and their sensitivity to γ-irradiation was examined. Also, using siRNA, we evaluated the radiosensitization effect of *BACE2* inhibition on cancer cells, which show high *BACE2* expression levels.

METHOD

Genetic alteration of the *BACE1* gene in cancer cases



Expression Atlas, http://www.ebi.ac.uk/gxa/

Fig. 1 Structural organization and subcellular localization of BACE1 and BACE2. SP = signal peptide; Pro = pro-domain; DTG, DSG = catalytic domains; TM = transmembrane domain; C = C-terminal domain.

Farris F, Matafora V, Bachi A. The emerging role of β-secretases in cancer. J Exp Clin Cancer Res. 2021 Apr 29;40(1):147. doi: 10.1186/s13046-021-01953-3. PMID: 33926496; PMCID: PMC8082908.

- **siRNA of *BACE1* & *BACE2***
  - Silencer Select Validated siRNA targeting *BACE1* (s24218, Life Technologies)
  - SiRNA2 (hs.Ri.*BACE1*.13.2,INTEGRATED DNA TECHNOLOGIES)
  - SiRNA1 (INTEGRATED DNA TECHNOLOGIES)
  - SiRNA2 (INTEGRATED DNA TECHNOLOGIES)
- **mRNA level measurement**

Cells → Reverse Transcription → Real-time PCR

- **Clonogenic survival assay**

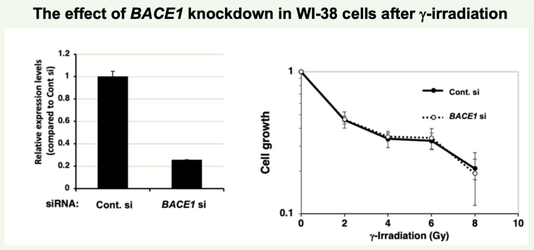
Cells → γ-irradiation → Colony formation

- **MTT assay**
- **γ-Irradiation**
- **Western blot analysis**

CCK Assay (Dojindo)
^{137}Cs γ-Irradiator (Gammacell 220, Nordion, Canada)
^{137}Cs γ-emitting irradiator (PS-3100SE, Pony Industry, Osaka, Japan)
Dose rate :1.0 Gy/min

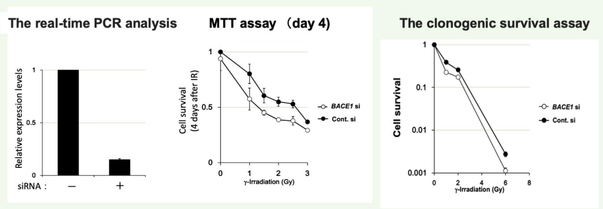
RESULTS & DISCUSSION

The effect of *BACE1* knockdown in WI-38 cells after γ-irradiation



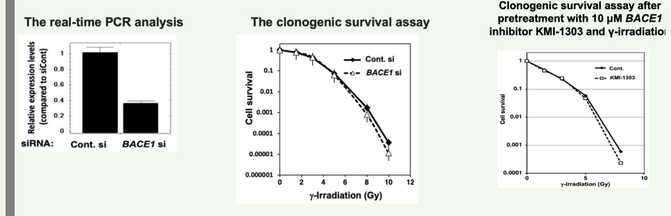
The knockdown of *BACE1* did not show changes in radiosensitivity in normal fibroblast WI-38 cells.

***BACE1* knockdown resulted in the radiosensitization of T-Rex HeLa cells**



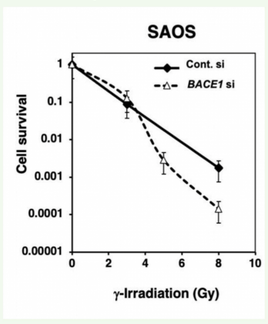
The mRNA expression of *BACE1* decreased to approximately 15% of the control level two days after transfection.*BACE1* knockdown enhanced the sensitivity of T-Rex HeLa cells to γ-irradiation.

Radiosensitization effect of *BACE1* knockdown and *BACE1* Inhibitor in U2OS cells, which have normal p53 function



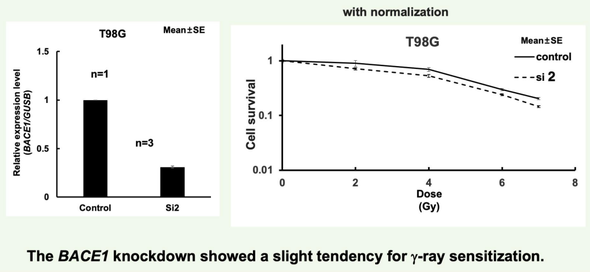
The clonogenic survival assay revealed that the sensitizing effect of *BACE1* knockdown was observed at higher dose ranges of 6–10 Gy.

The effect of *BACE1* knockdown on sensitivity to γ-irradiation in SAOS cells, which have p53 mutation



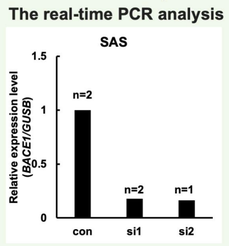
BACE1 knockdown exhibited a γ-ray sensitizing effect at higher doses of 4 Gy or more, compared to U2OS cells.

The effect of *BACE1* knockdown on sensitivity after γ-irradiation in T98G cells, which have p53 mutation

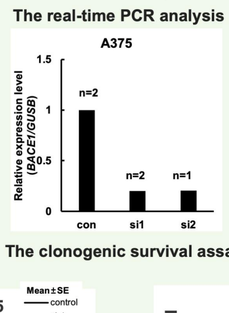


The *BACE1* knockdown showed a slight tendency for γ-ray sensitization.

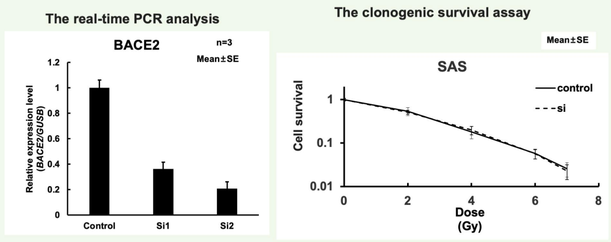
The effect of *BACE1* knockdown after γ-irradiation in SAS cells



The effect of *BACE1* knockdown after γ-irradiation in A375 cells



The effect of *BACE2* knocked down with siRNA after γ-irradiation in SAS cells



BACE2 knockdown did not show a sensitizing effect to γ-ray irradiation in SAS cells. Further investigation is required to understand the effects of *BACE2* knockdown.

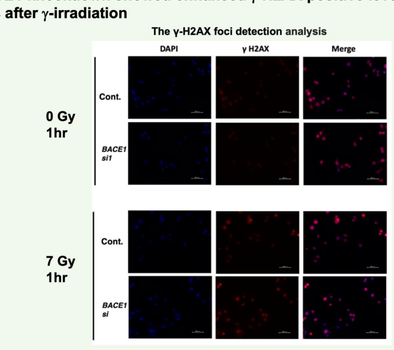
Unpublished, do not post

The effect of *BACE1* knockdown on sensitivity after γ-irradiation in T98G cells, which have p53 mutation

The *BACE1* knockdown showed a slight tendency for γ-ray sensitization.

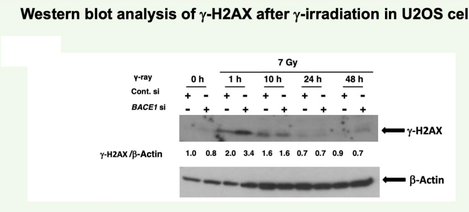
The effect of *BACE1* knockdown after γ-irradiation in A375 cells

***BACE1* knockdown showed enhanced γ-H2AX positive levels in T-Rex HeLa cells after γ-irradiation**



In the *BACE1* knockdown group, a higher γ-H2AX foci level was observed one hour after 7 Gy irradiation compared to the control group.

Western blot analysis of γ-H2AX after γ-irradiation in U2OS cells



BACE1 knockdown transiently enhanced the increase of the double-strand DNA break marker γ-H2AX in U2OS cells.

Cell line	Cell Type	p53 pathway aberration	Radiosensitization by *BACE1* knockdown
T98G	Human glioblastoma	+	+
SAOS	Human osteosarcoma	+	+ +
HeLa	Human cervical cancer	+	+ +
SAS	Human oral squamous cancer	-	-
WI38	Human normal fibroblast	-	-
U2OS	Human osteosarcoma	-	+
A375	Human malignant melanoma	-	-

Unpublished, do not post

CONCLUSION

No sensitizing effect to γ-ray irradiation was observed in the normal fibroblast cell line WI-38. *BACE1* knockdown transiently enhanced the increase of the double-strand DNA break marker γ-H2AX in U2OS cells. *BACE1* knockdown showed a higher tendency of sensitizing effect in p53-deficient cell lines compared to cell lines with normal p53 function.

These results suggest that *BACE1* may be a potential target for radiation sensitization in particular cancer cells.

FUTURE WORK / REFERENCES

Currently, we are investigating the γ-ray sensitizing effects of *BACE1* knockdown in other cancer types, as well as the radiation sensitization effects through the inhibition of *BACE2*, which is known to be overexpressed in particular cancers.