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研究課題名（和文）Induction of Paraptosis: A novel strategy to overcome radiation resistance in cancer cells

研究課題名（英文）Induction of Paraptosis: A novel strategy to overcome radiation resistance in cancer cells

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研究成果の概要（和文）：本研究ではHCT-116大腸癌細胞においてバルドキソロンメチル(CDDO-Me)はパラプトーシスを誘導し、細胞質空胞化を促進する。抗酸化物NACはCDDO-MeによるROSの誘導および細胞質の空胞化を抑制し、新しい細胞死（パラプトーシス）においてROS産生の重要性を示す。パラプトーシス阻害剤であるAlix/AIP-1がCDDO-Meによるパラプトーシス抑制することを明らかにした。また、タンパク質阻害剤であるシクロヘキシミドはCDDO-Meによる空胞化および細胞死を阻害した。HCT-116細胞においてCDDO-Me のROSの生成およびERストレスを介したパラプトーシスは潜在的な治療作用を示す。

研究成果の学術的意義や社会的意義
Benefits of Selective cancer cell killing with lesser side effects are obvious on the quality of life in cancer patients. The determination of novel agents which can induced cell death particularly in cancer and protect normal tissue will help to provide a new paradigm in the field of oncology.

研究成果の概要（英文）：In this study, we report for the first time that bardoxolone methyl (CDDO-Me), induces paraptosis in HCT-116 colorectal cancer cells, by promoting vacuolation that results from the endoplasmic reticulum (ER). Induction of ROS by CDDO-Me triggers ER-stress signaling, as paraptosis with cytoplasmic vacuolation could be blocked by an antioxidant N-acetylcysteine (NAC). Mechanistic investigation revealed that the indigenous level of known paraptosis inhibitor, Alix/AIP-1 was down-regulated by CDDO-Me treatment. Besides, Cycloheximide prevents CDDO-Me-induced vacuolation and cell death, indicating the requirement of active de-novo protein synthesis. Notably, this cytoplasmic vacuolation was brought out by a lack of caspase activation or PARP cleavage and DNA fragmentation. CDDO-Me persistently induces the expression of autophagy marker LC3-II, along with ER stress markers, Bip, and CHOP. CDDO-Me mediated paraptosis in HCT-116 cells show potential therapeutic effects.

研究分野：放射線科学関連

キーワード：Paraptosis　Radiation　Oxidative stress

１．研究開始当初の背景

Cancer cells tend to develop dysregulation in apoptosis to promote their survival and ultimately lead to chemo or radio-resistance. Recently, the induction of non-apoptotic cell death such as paraptosis, non-autophagic cytoplasmic vacuolation death has been reported as an alternative strategy to improve therapy resistance. Radiotherapy continues to play an important role in the cancer treatment. Despite these advancements the use of radiation therapy is linked with serious concerns and fails to eradicate tumors, especially in case of radio-resistant tumors. Apoptosis resistance is often develops in cancer cells and is one of key hallmarks for treatment failure.
Given the increasing problem of resistant to apoptosis, the search for alternatives means to induce cancer cell death are urgently required. Paraptosis is a type of program cell death that is characterized by vacuolation related to dilation of endoplasmic reticulum (ER) and/or mitochondria. Its underlying mechanism is clearly distinct from apoptosis. Both increased oxidative stress and ER-stress related paraptosis induction has been reported (Jeng-Yuan Shiau *et al.* Oncotarget 2017 and Lin Wang *et al.* Cell Death and Disease 2017). Therefore, in this study the aim was to create a cancer cell death enhancement method by utilizing drugs/chemical modifiers of intracellular redox signaling that induces distinct cancer cell death alone or with radiation to improve therapy resistance.

２．研究の目的

The purpose of this study is to clarify the mechanism of enhancement of intracellular oxidative stress, underlying mechanism to induce paraptosis or non-autophagic cancer cell death and sensitization by novel chemical agents. In this project we focused on the chemical agents that possess dual effects i) must be selective to cancer cells ii) generate intracellular oxidative stress mediated switch in cell death pathways. We used the derivative of synthetic triterpenoids Bardoxolone methyl (CDDO-Me), (which inhibits NF-κB activity in cancer cells, promotes intracellular ROS production and decrease gluthathione level). CDDO-Me target cancer cells specifically and protects normal cell from radiation (Ashmawy EI M *et al*, PLoS ONE, 2014). In human colorectal cancer HCT-116 cells we identify that marked potentiation of oxidative stress and glutathione inhibition by CDDO-Me alone causes ER stress mediated induction of paraptosis. It would contribute to the treatment of resistance cancer and in the development of effective therapeutic strategy.

３．研究の方法

The effect of bardoxolone methyl on HCT-116 cells were studied. A number of assays were employed to investigate molecular pathway of paraptosis.

1. Assays relevant to cell death: cell viability, tryphan blue, DNA fragmentation, Anexxin V-FITC/PI double staining, cell cycle analysis.
2. Morphological changes: Giemsa staining, colony formation assay, microscopy.
3. Molecular mechanism: ROS detection (Intracellular H_2O_2, DCFH-DA staining), Intracellular GSH decrease, Intracellular calcium release, loss of mitochondrial potential (TMRM staining).
4. Cell death type confirmation: western blot for caspase-3, PARP, LC-III, p62, Beclin, BiP and CHOP, apoptosis inhibitor Z-VADFMK, autophagy inhibitor 3MA.
5. Paraptosis confirmation: Alix protein expression and cycloheximide (protein synthesis inhibitor).
6. Gene chip analysis to identify molecular targets.

４．研究成果

In this project, in our preliminary experiment the combination of bardoxolone methyl (CDDO-Me) with radiation does not show any enhancement in cell death or induction of distinct cell death type. However, interestingly CDDO-Me alone treatment shows vacuole mediated cell death in HCT-116 cells. Therefore, the subsequent experiments were carried out using CDDO-Me alone treatment.

1. CDDO-Me treatment (0–500 nM) does not induce increase in the DNA fragmentation and changes in apoptosis. However, CDDO-Me treatment decreased the cell viability of HCT-116 cells in a concentration-dependent manner (Fig.1 A, B and C).

Fig.1 A

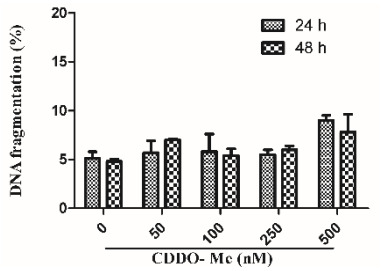


Fig.1 B

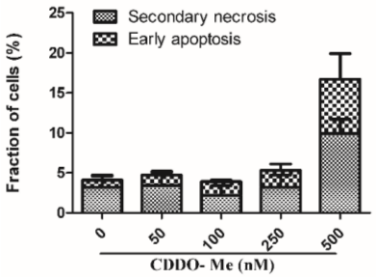
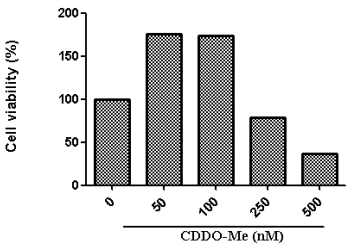


Fig.1 C



2. CDDO-Me treatment for 48 hr induced morphological changes with vacuole formation. At 250 nM cytoplasmic vacuole start to appear and at 500 nM more prominent vacuoles were seen. The colony formation assay also showed decreased cell proliferation at 250 and 500 nM Fig. 2 A, B. CDDO-Me 500 nM used for subsequent experiments.

Fig.2 A

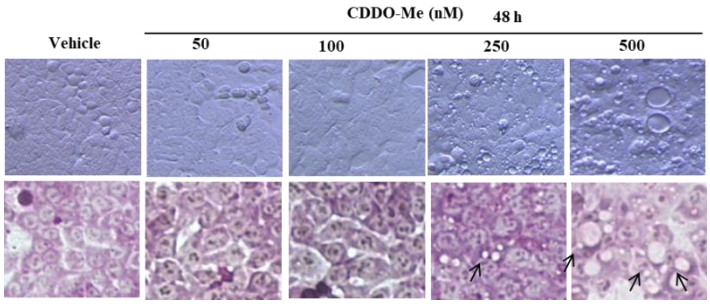
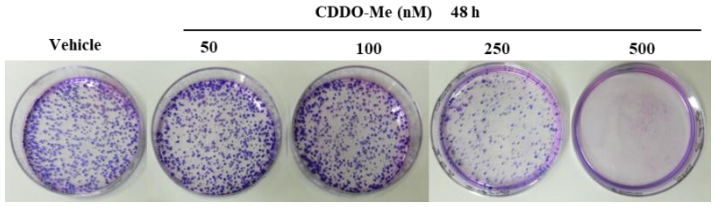


Fig.2 B



3. CDDO-Me induced cytoplasmic vacuolation in HCT-116 cells was not prevented in the presence of ZVAD-FMK apoptosis inhibitor and 3-methyladenine (3-MA) autophagy inhibitor. Failure of Z-VAD and 3-MA to protect cells suggest the non-autophagic cytoplasmic vacuolation in HCT-116 by CDDO-Me. In particular, this cytoplasmic vacuolization is independent to caspase-3 activation, PARP cleavage and autophagy activation Fig.3 A, B and C.

Fig.3 A

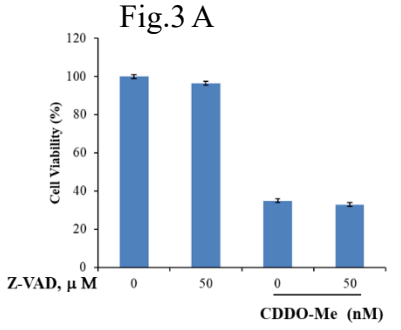


Fig.3 B

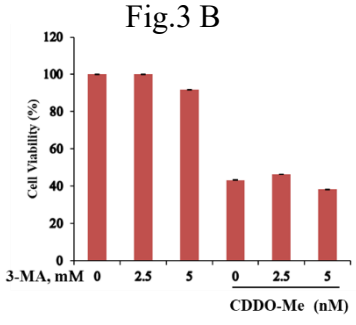
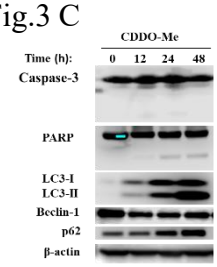


Fig.3 C



4. Cycloheximide (CHX), protein synthesis inhibitor pretreatment blocked vacuolization in response to CDDO-Me. The expression of Alix protein, which is a known inhibitor of paraptosis also downregulated following treatment. Fig 4 A, B. This suggest that paraptosis is a major cell death in HCT-116 cells treated with CDDO-Me.

Fig.4 A

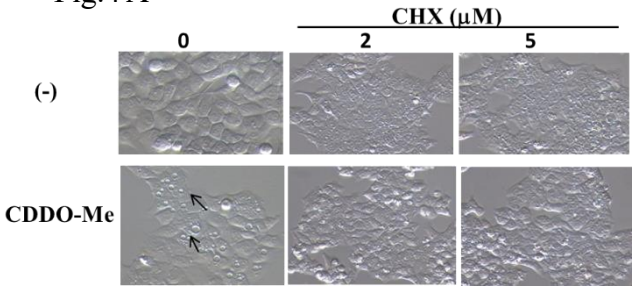
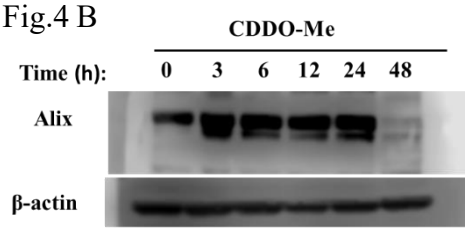
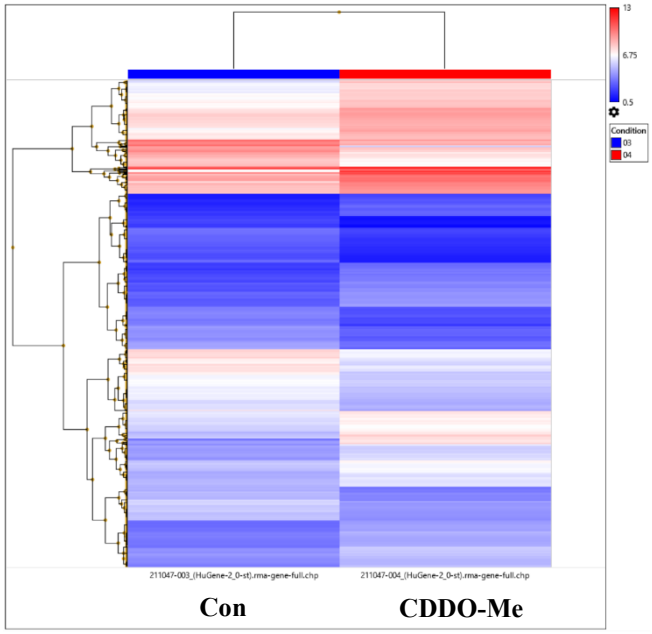


Fig.4 B



5. In this study, we have planned to perform *in-vivo* experiments in tumor bearing mouse. However, based on our interesting results the gene chip analysis was performed to identify the molecular target of paraptosis. Several upregulated or downregulated genes were identified in CDDO-Me treatment compared to control Fig. 5.

Fig. 5



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５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Rehman MU, Zhao QL, Refaat A, Jawaid P, Sakurai H, Saitoh JI, Kondo T, Noguchi K.
２．発表標題 Bardoxolone methyl (CDDO-Me or RTA402) induces paraptosis through reactive oxygen species-mediated endoplasmic stress and intracellular calcium release in human colorectal cancer HCT-116 cells
３．学会等名 26th Annual meeting of the Society of Redox Biology and Medicine (SFRBM 2019)（国際学会）
４．発表年 2019年

１．発表者名 Rehman MU, Zhao QL, Refaat A, Jawaid P, Sakurai H, Kondo T, Noguchi K.
２．発表標題 Reactive oxygen species-mediated stress and release of Ca 2+ trigger CDDO-Me induced non-apoptotic/non-autophagic cytoplasmic vacuolation death in colorectal cancer.
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２．発表標題 Bardoxolone methyl (CDDO Me) induces paraptosis in colorectal cancer cells via ROS mediated activation of ER stress signaling.
３．学会等名 The 25th Cancer Therapy Sensation Research Group, International Association of Sensitization for Cancer Treatment Symposium (IASCT).（国際学会）
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〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関