様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月　 ５ 日現在

機関番号：１３３０１
研究種目：若手研究
研究期間：2021～2023
課題番号：２１Ｋ１５５０２

研究課題名（和文）The role of activin combined with different gene mutations in colorectal cancer EMT

研究課題名（英文）The role of activin combined with different gene mutations in colorectal cancer EMT

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交付決定額（研究期間全体）：（直接経費）　　3,600,000円

研究成果の概要（和文）：この課題は、TGFβシグナルに関わる遺伝子変異の同定を目指した。Kras変異は、アクチビンAの細胞増殖抑制を抑制した。さらに、Trp53の機能獲得変異（GOF）は、LOHによる野生型Trp53の欠損で、アクチビンAで誘導されるpartial EMTを促進した。RNAシークエンスの結果から、Trp53のGOF/LOHの細胞では、Hmga2の発現が上昇し、HMGA2の欠損は、Trp53 GOF/LOH細胞におけるアクチビンAが誘導するpartial EMTを抑制した。これらの結果は、TP53 GOF/LOHが、TGFβが誘導する大腸癌の悪性化に関わる重要な遺伝子変異であることを示している。

研究成果の学術的意義や社会的意義
In this project, we clarified the function and mechanism of activin in colon cancer. The findings indicate that activin signaling may be an effective therapeutic target for colorectal cancer harboring TP53 GOF mutations and expand our knowledge about EMT mechanism.

研究成果の概要（英文）：This project focuses on identifying genetic mutations that determine the outcome of TGFβ signaling. KrasG12D mutation protected organoid cells from activin A (TGFβ superfamily cytokine)-induced growth suppression by inhibiting p21 and p27 expression. Furthermore, Trp53R270H gain-of-function (GOF) mutation together with loss of wild-type Trp53 by loss of heterozygosity (LOH) promoted activin A-induced partial EMT and increased metastatic incidence. RNA sequencing analysis indicated that expression of Hmga2 was significantly upregulated in organoids with Trp53 GOF/LOH alterations. Importantly, loss of HMGA2 blocked activin A-induced partial EMT and metastasis in Trp53 GOF/LOH organoids. These results indicate that TP53 GOF/LOH is a key genetic state that primes for TGFβ family-induced partial EMT and malignant progression of colorectal cancer. Activin signaling may be an effective therapeutic target for colorectal cancer harboring TP53 GOF mutations.

研究分野：cancer biology

キーワード：activin　driver gene mutation　colorectal cancer　partial EMT

様　式　Ｃ－１９、Ｆ－１９－１（共通）

１．研究開始当初の背景

The expression level of activin (Staudacher et al., Sci Rep, 2017) and its' receptor (Liu et al., Biochem Biophys Res Commun, 2016) are significantly high in malignant colorectal cancer. Moreover, TCGA data indicate that high expression of activin A subunit (INHBA) corresponds to poor survival of the patient in CRC. In lung adenocarcinoma activin mediates platinum chemotherapy resistance through TGFß-activated kinase 1 (TAK1) (Marini et al., Sci Transl Med, 2018) and activin A promotes squamous carcinogenesis via fibroblast reprogramming (Cangkrama et al., EMBO Mol Med, 2020). However, the role of activin in colorectal cancer development has not been yet elucidated. The majority of deaths from tumors including CRC are caused by metastases (Dillekås et al., Cancer Med, 2019). It shows that activin A signaling promotes melanoma metastasis through immune evasion (Donovan et al., J Invest Dermatol, 2017). However, in CRC, the role and mechanism of activin in promoting and maintaining metastasis are unclear. Therefore, understanding the role of activin in colorectal cancer will help develop better treatment strategies for colorectal cancer.

In CRC, Apc, Kras, Tgfbr2, p53 and Fbxw7 are frequently mutated genes and accumulation of those mutations causes tumor development and gradual malignant progression of cancer cells. (Sakai et al., Cancer Res, 2018). The function of TGFß in tumor is related to the malignancy of the tumor (Colak et al., Trends Cancer, 2017), as a member of TGFß superfamily, however, the role of activin in different malignant tumors and the relationship between activin and the key driver gene mutations (Apc, Kras, Tgfbr2, p53 and Fbxw7) are still unclear. Therefore, understanding the correlation between activin and key driver gene mutations is necessary for choosing appropriate treatment strategies for individual patient.

２．研究の目的

Since activin shares the same downstream genes as TGFß, we hypothesized that activin induces EMT in colorectal cancer, even in the tumors with genetic alteration in TGFß receptor genes. Moreover, since normal and tumor cells respond differently to the TGFß signaling, some gene mutations in carcinogenesis may contribute to activin's conversion of its induction of apoptosis to induction of EMT. In this project, we will determine the combination of genetic switching to respond to activin for EMT induction and the responsible molecules under activin signaling for EMT. Through our research findings, we try to find a new treatment strategy and verify its inhibitory effect on metastasis.

３．研究の方法

In order to know the role of activin in different stages of colorectal cancer and confirm which driver mutation(s) regulate resistance to activin-induced differentiation/cell death and induction of activin-induced EMT phenotype, I plan to conduct the following experiments:

1. Prepare and culture the intestinal tumor-derived organoids with multiple gene mutations (ex. A, AK, AKP, AKT, AKTP, AKTPF etc). For *Trp53* mutations, we have three different genotypes, i.e., -/-, +/R273H, and R273H/R273H. In our laboratory (Cancer Research Institute, Kanazawa

University), organoids with these multiple gene mutations have been established and their metastatic abilities have been characterized.

2. Treat these organoids with activin in collagen gel culture conditions.

3. Then, the morphological changes will be examined using a dissection microscope, and immunocytochemistry will be performed to examine Smad activation using confocal microscope. The cell death will be examined using EdU labeling and examination of apoptosis markers. Based on these experiments, we will determine the role of activin in different stages (genotypes) of CRC and the candidate key driver gene mutations will be revealed, which is associated with activin-induced EMT.

4. RNA sequencing will be performed for selected organoids that are resistant or sensitive to activin-induced EMT and their genetic variant organoids. By analyzing the data, the potential mechanism by which interaction of activin and key driver gene mutations induces EMT will be revealed.

5. Activin-induced EMT-morphological changes will be examined to confirm the mechanism after disruption of the candidate gene (found in step 4) by using CRISPR/Cas9 system.

6. If candidate molecules for regulation of activin-induced EMT are identified, liver metastasis will be examined by transplantation of organoids with candidate gene disruption (generated in step 5). If inhibitors of candidate molecules are available, they will be studied in the in vivo study to find new strategies against CRC metastasis.

４．研究成果

In this project, the organoids with different combination of driver gene mutations were simulated with activin and the cell survival and morphological changes were examined.

For cell survival: using EdU experiments to examine the proliferation ratio, we found that the organoids with Kras mutation could survive well after activin induction, otherwise, the organoids without Kras mutation go through apoptosis. Furthermore, blocking Kras-MEK signaling pathway by MEK inhibitors, the organoids with Kras mutation go through apoptosis under activin-induction. Mechanically, Kras mutation downregulated p21 and p27 (the downstream genes of TGFβ) to suppress activin-inducted apoptosis.

For morphological changes: after activin treatment, the morphological changes were examined using a dissection microscope, and immunocytostaining was examined using confocal microscope. We found that the organoids with p53 homozygous mutation instead of heterozygous mutation form EMT-like morphology. Importantly, we confirmed that activin induced the partial EMT in tumor-derived organoid. Furthermore, overexpression of wt-p53 in the organoids with p53 homozygous mutation inhibits activin-induced morphological; on the other hand, mutated p53 overexpression in $AKTP^{null}$ organoids enhanced activin-inducted partial EMT phenotype. These results confirmed that p53 gain-of-function mutation with lose wild-type p53 regulates activin-induced partial EMT.

In vivo: to confirm activin-induced partial EMT associated with metastasis, we pretreated the organoids with p53 homozygous mutation and injected into tail vein of the mice. By examining lung metastasis, we found that activin treated organoids form more lung metastasis. Furthermore, we revealed that activin treatment promotes colonization of tumor cells in lung to enhance lung metastasis.

For mechanism: to clarify the mechanism by which p53 interacts with activin to induce EMT-like morphological changes, we performed RNA-seq. RNA sequencing analysis indicated that expression of Hmga2, encoding a cofactor of the SMAD complex that induces EMT transcription factors, was

significantly upregulated in organoids with Trp53 GOF/LOH alterations. Importantly, loss of HMGA2 suppressed expression of Twist1 and blocked activin A–induced partial EMT and metastasis in Trp53 GOF/LOH organoids. These results indicate that TP53 GOF/LOH is a key genetic state that primes for TGFß family-induced partial EMT and malignant progression of colorectal cancer. Activin signaling may be an effective therapeutic target for colorectal cancer harboring TP53 GOF mutations.

These findings were published on **Cancer Research** (Wang, D., Nakayama, M., Hong, C. P., Oshima, H., & Oshima, M. (2024). Gain-of-Function p53 Mutation Acts as a Genetic Switch for TGFß Signaling–Induced Epithelial-to-Mesenchymal Transition in Intestinal Tumors. Cancer Research, 84(1), 56-68.) and the result image of this article was selected as the cover image of "Cancer Research Vol. 84 Issue 1".

With the support of this grant, another related work of ours was published on **Small** (Wang, D., Nguyen, H. G., Nakayama, M., Oshima, H., Sun, L., Oshima, M., & Watanabe, S. (2023). Mapping nanomechanical properties of basal surfaces in metastatic intestinal 3d living organoids with high‐speed scanning ion conductance microscopy. Small, 19(9), 2206213.).

５．主な発表論文等

〔雑誌論文〕　計6件（うち査読付論文　4件／うち国際共著　1件／うちオープンアクセス　3件）

１．著者名 Wang Dong、Nakayama Mizuho、Hong Chang Pyo、Oshima Hiroko、Oshima Masanobu	４．巻 84
２．論文標題 Gain-of-Function p53 Mutation Acts as a Genetic Switch for TGFβ Signaling-Induced Epithelial-to-Mesenchymal Transition in Intestinal Tumors	５．発行年 2023年
３．雑誌名 Cancer Research	６．最初と最後の頁 56～68
掲載論文のDOI（デジタルオブジェクト識別子） 10.1158/0008-5472.CAN-23-1490	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Wang Dong、Nguyen Han Gia、Nakayama Mizuho、Oshima Hiroko、Sun Linhao、Oshima Masanobu、Watanabe Shinji	４．巻 19
２．論文標題 Mapping Nanomechanical Properties of Basal Surfaces in Metastatic Intestinal 3D Living Organoids with High‐Speed Scanning Ion Conductance Microscopy	５．発行年 2022年
３．雑誌名 Small	６．最初と最後の頁 2206213～2206213
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/smll.202206213	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Morita Atsuya、Nakayama Mizuho、Wang Dong、Murakami Kazuhiro、Oshima Masanobu	４．巻 114
２．論文標題 Frequent loss of metastatic ability in subclones of Apc, Kras, Tgfbr2, and Trp53 mutant intestinal tumor organoids	５．発行年 2023年
３．雑誌名 Cancer Science	６．最初と最後の頁 1437～1450
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/cas.15709	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

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２．論文標題 Genetic Alterations and Microenvironment that Drive Malignant Progression of Colorectal Cancer: Lessons from Mouse and Organoid Models	５．発行年 2022年
３．雑誌名 Journal of Cancer Prevention	６．最初と最後の頁 1～6
掲載論文のDOI（デジタルオブジェクト識別子） 10.15430/JCP.2022.27.1.1	査読の有無 有
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1．著者名 Wang Dong、Sun Linhao、Okuda Satoru、Yamamoto Daisuke、Nakayama Mizuho、Oshima Hiroko、Saito Hideyuki、Kouyama Yuta、Mimori Koshi、Ando Toshio、Watanabe Shinji、Oshima Masanobu	4．巻 280
2．論文標題 Nano-scale physical properties characteristic to metastatic intestinal cancer cells identified by high-speed scanning ion conductance microscope	5．発行年 2022年
3．雑誌名 Biomaterials	6．最初と最後の頁 121256～121256
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.biomaterials.2021.121256	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

1．著者名 Yamamoto Daisuke、Oshima Hiroko、Wang Dong、Takeda Haruna、Kita Kenji、Lei Xuelian、Nakayama Mizuho、Murakami Kazuhiro、Ohama Takashi、Takemura Hirofumi、Toyota Mutsumi、Suzuki Hiromu、Inaki Noriyuki、Oshima Masanobu	4．巻 257
2．論文標題 Characterization of RNF43 frameshift mutations that drive Wnt ligand- and R-spondin-dependent colon cancer	5．発行年 2022年
3．雑誌名 The Journal of Pathology	6．最初と最後の頁 39～52
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/path.5868	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

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1．発表者名 Dong Wang, Mizuho Nakayama, Hiroko Oshima, Masanobu Oshima
2．発表標題 Activin promotes intestinal tumor cell metastasis by interacting with gain-of-function mutant p53
3．学会等名 The 81th annual meeting of the Japanese cancer association（国際学会）
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1．発表者名 Dong Wang, Mizuho Nakayama, Hiroko Oshima, Masanobu Oshima
2．発表標題 Gain-of-function p53 mutation with LOH promotes activin A-induced partial EMT and metastasis potential
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4．発表年 2023年

〔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