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研究課題名（和文）The role of activin signaling in colorectal cancer EMT induction

研究課題名（英文）The role of activin signaling in colorectal cancer EMT induction

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研究成果の概要（和文）：アクチビンと大腸がんドライバー遺伝子変異の相互作用が、細胞のアポトーシスとEMT誘導の関与について検討した。様々なドライバー遺伝子変異を持つオルガノイドをアクチビンで刺激した結果、Kras変異がアクチビン誘導アポトーシスを抑制すること、さらに、アクチビンが変異型p53遺伝子との相互作用でEMTを誘導することがわかった。また、アクチビンで刺激したオルガノイドの移植は、刺激してないオルガノイドより多くの肺転移巣を形成した。RNA-seqの結果から、アクチビンと変異型p53遺伝子で、いくつかのシグナル経路が誘導されることがわかった。これらの結果から、新たな候補阻害剤を検索できる可能性を示唆した。

研究成果の学術的意義や社会的意義
In this project, we explored the role and mechanism of activin in colorectal cancer EMT. We found that activin induces EMT by interacting with Kras and p53 mutations, which expands our knowledge about EMT mechanism and contributes to the development of anti-cancer drugs.

研究成果の概要（英文）：In order to know the role of activin in colorectal cancer EMT and which driver gene mutations contribute to activin's conversion from induction of apoptosis to induction of EMT, the organoids with different combination of driver gene mutations were simulated with activin and the cell survival and EMT were examined. Cell survival: by using activin simulation and blocking Kras-MEK signaling, we found that Kras mutation inhibits activin-induced apoptosis. Morphological changes: we found that activin induces EMT via interacting with p53 homozygous mutation. In vivo: activin pretreated organoids form more lung metastasis. For mechanism: by using RNA-seq, we found that under the regulation of activin and p53 homozygous mutation, some signaling pathways, such as Wnt, p38-MAPK, Stat3 signaling, were upregulated. According to these findings we will be able to test some inhibitors for inhibiting the morphological changes and the candidate inhibitors may be used for clinical trial.

研究分野：cancer biology

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

１．研究開始当初の背景(Background at the beginning of the Research)

The role of TGFβ has been well studied, which induces differentiation and apoptosis of normal epithelial cell and induces tumor malignancy (Massagué, et al., **EMBO J**, 2000). In CRC, the malignant progression depends on the accumulation of mutations in driver genes such as *APC*, *KRAS*, *TGFBR2*, *TP53* and *FBXW7*, and more than three mutations are required for the acquisition of metastatic ability (Sakai et al., **Cancer Res**, 2018). CRC is the second leading cause of cancer-related death in 2018 provided by WHO, and metastasis is responsible for decreased survival of cancer patients (Chaffer et al., **Science**, 2011). During metastasis process, EMT (Epithelial–mesenchymal transition) is an important intermediate process for invasion step, and inhibition of EMT may reduce tumor metastasis (Aiello et al., **Nature**, 2017). TGFβ has been the most characterized cytokine to induce EMT, however, 30% CRC carry mutation in TGFβ pathway including TGFβ receptor type 2 (*TGFBR2*) that do not respond to TGFβ ligand (Bella et al., **Cancer Treat Res**, 2010). Activin contains three variants (the most studied is activin A, which is composed of two INHBA subunits), all of which are secretory ligands of TGFβ family (Loomans et al., **Cancers**, 2015). As activin shares the same downstream molecules, Smad2/3, with TGFβ (Wamsley et al., **Cancer Res**, 2015), activin may induce EMT in these TGFβ-impaired cancer cells. Although a few studies indicated that activin induces EMT (Bauer et al, **Mol Cancer**,2015), the mechanism has not been yet elucidated.

２．研究の目的(Purpose of the Research)

Since activin shares the same downstream genes as TGFβ, we hypothesized that activin induces EMT in colorectal cancer, even in the tumors with genetic alteration in TGFβ receptor genes. Moreover, since normal and tumor cells respond differently to the TGFβ 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(Research Methods)

In order to know which downstream molecules or signal pathway interacts with activin-Smad3/4 to induce EMT and which gene mutations in CRC carcinogenesis contribute to activin's conversion from induction of apoptosis to induction of EMT, I plan to conduct the following experiments:

1. Prepare and culture organoids with multiple gene mutations. In our lab, organoids with multiple gene mutations have been established and their metastatic abilities have been characterized.
2. Treat these organoids with activin in collagen gel.
3. After activin stimulation, the morphological changes will be examined using a dissection microscope, and immunocytostaining will be examined using confocal microscope.

4. Activin treatment studies will be performed to all available organoids with variety of genotypes.

5. Through the steps 3 and 4, I will figure out which genetic mutations are required for activin-induced EMT. RNA sequencing and functional screening using CRISPR/Cas9 system in organoids will be used to elucidate the regulatory mechanism underlying activin-induced EMT.

6. If candidate molecules for activin that induces EMT are identified, liver metastasis will be examined by transplantation of KO organoids (generated in step 5) to find new strategies for reducing CRC metastasis.

４．研究成果(Research results)

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, 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. This result suggest that Kras mutation inhibits activing-induced cell 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. Furthermore, overexpression of wt-p53 in the organoids with p53 homozygous mutation inhibits activin-induced morphological, which suggests that p53 homozygous mutation interacts with activin to induce EMT-like morphological changes.

In vivo: to confirm activin-induced morphological changes 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.

For mechanism: to clarify the mechanism by which p53 interacts with activin to induce EMT-like morphological changes, we performed RNA-seq and we found that after activin simulation, some signaling pathways, such as Wnt, p38-MAPK, Stat3 signaling, were significantly upregulated. According to these findings we will be able to test some inhibitors for inhibiting the morphological changes and the candidate inhibitors may be used for clinical trial.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔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