新学術創成研究機構若手 PI

Inflammation and Epithelial Plasticity

上皮可塑性・炎症ユニット

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Graduate Student　Zachary YONG (D3) (Co-supervisor: Prof. Masanobu Oshima)

【 Abstract 】

The link between inflammation and cancer has long been established. A proinflammatory tumor microenvironment is generally regarded as one that promotes carcinogenesis, tumor growth and the suppression of tumor immunity. Although great advances have been made in terms of the cellular composition and immune interaction within a tumor niche, how cell-intrinsic mutations within the epithelium drive this process, through the aberrant secretion of growth factors and cytokines, is under appreciated. We are interested in two specific aspects of this interaction: 1) the role of epithelial-derived IL23A in modulating immunity during gastrointestinal infection and carcinogenesis; and 2) the increased epithelial plasticity during gastrointestinal inflammation and repair.

＜2019 research achievement and future plan＞

Following the successful completion of our collaborative work with Prof Oshima's group on the oncogenic activities of miR-135b in inflammation-associated gastric carcinogenesis, we resumed our investigation on the regulation and function of epithelial cell-derived IL23A. In intestinal epithelial cells, we found that IL23A is secreted in a novel, IL12B-independent form, which is strongly driven by mitogenic and inflammatory signals. We believe this means IL23A has an independent function from canonical IL-23 (IL23A/IL12B), and contributes a distinct immune signal during intestinal inflammation, regeneration, and possibly carcinogenesis. Accordingly, we observed that certain MAPK mutant CRC lines have constitutively high *IL23A* expression, which could be targeted by MEK1/2 and/or NF-κB pathway inhibitors. The use of these inhibitors also revealed a strong cooperativity between mitogenic and inflammatory signals. To understand this, we interrogated the *IL23A* promoter and found that these signals cooperate at the promoter level through the assembly of a promoter enhancer complex consisting of c-Jun, RUNX3/1 and RelA/p65. This novel mechanism provides a satisfactory explanation to the cooperativity of the agonists and pharmacological inhibitors we observed. A manuscript that reports these novel observations is currently under revision. In the coming year, our focus shall be on elucidating the immune functions of epithelial-derived IL23A, especially in the interaction with primary innate lymphoid cells (ILCs) and proinflammatory

IL-17-secreting T lymphocytes. In addition, we shall be investigating the potential role of epithelial-derived IL23A in modulating tumor immunity and tumor growth through the xenograft models we have established this year.

【 Achievements 】

＜Publications (Primary)＞

1. Han TS, **Voon DC***, Oshima H, Nakayama M, Echizen K, Sakai E, Yong ZWE, Murakami K, Yu L, Minamoto T, Ock CY, Jenkins BJ, Kim SJ, Yang HK, Oshima M*. (2018) *MicroRNA-135b acts downstream of Interleukin-1 signaling during inflammation-associated gastric carcinogenesis*. Gastroenterology. 2019 Mar;156(4):1140-1155.e4. *Joint Corresponding Author.

＜Publications (Collaboration)＞

1. Lim KS, Mohamed MS, Wang H, Hartono, Hazawa M, Kobayashi A, **Voon DC**, Kodera N, Ando T, Wong RW. (2020) *Direct visualization of avian influenza H5N1 hemagglutinin precursor and its conformational change by high-speed atomic force microscopy.* Biochim Biophys Acta Gen Subj. 2020 Feb;1864(2).

＜Symposiums (Oral Presentations)＞

1. **Voon DC.** *An emerging role for RUNX proteins in immune modulation via cytokine production*. The 22nd RUNX International Meeting. 18th Oct- 21st June 2019. Seoul, **Korea**. (Invited)

2. **Voon DC.** *An emerging role for RUNX proteins in immune modulation via cytokine production*. The 9th KU-CRI-FUSCC Joint Symposium on Cancer Biology. 3rd Sep 2019. Kanazawa, **Japan**.

3. **Voon DC.** *Inflammatory and mitogenic signals drive IL23A secretion independent of IL12B in intestinal epithelial cells*. 78th Annual Meeting of Japanese Cancer Association. 26-28th Sep 2019. Kyoto, **Japan**.

＜2019 research funds＞

＜Others Contribution＞

2019 9th FUSCC-CRIKU Joint Symposium Co-Coordinator

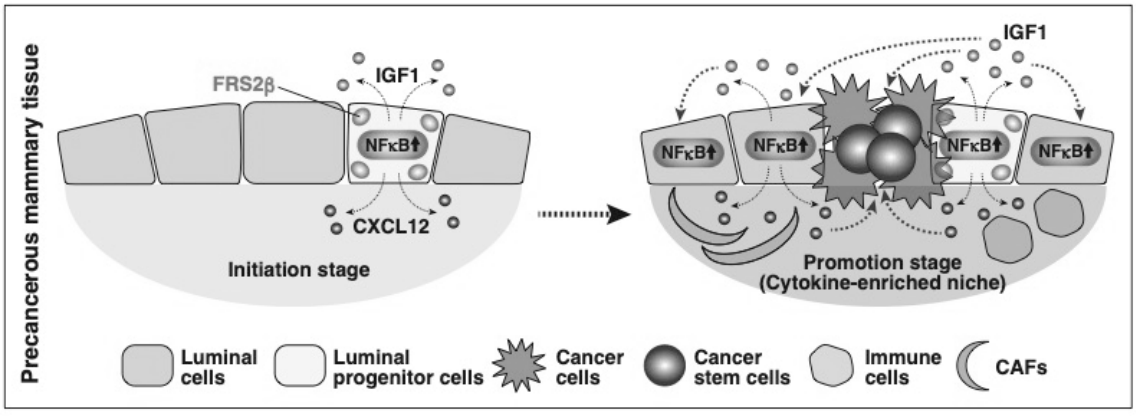
Microenvironment Regulation in Cancer Stem Cells
がん幹細胞環境制御ユニット

Assistant Professor　　Yasuto Takeuchi 竹内 康人

【 Abstract 】

Accumulating evidence indicates the presence of cancer stem-like cells (CSCs) in many types of tumors. They are defined as cell populations which have self-renewal ability and multi-differentiation capacity, and have been thought to contribute to tumor initiation and recurrence. Stem-cell properties are thought to be maintained in the CSC niche that is the tumor microenvironment surrounding CSCs. Therefore, our final goal is to identify key factors regulating the interaction between cancer stem-like cells (CSCs) and their niche.

We demonstrated that FRS2β was expressed in a small subset of luminal progenitor cells and induced the production of cytokines, including IGF1 and CXCL12, through activation of the NFκB pathway. IGF1 and CXCL12 amplify NFκB activation in surrounding mammary cells, leading to the establishment of a cytokine-rich microenvironment. This microenvironment supports the growth of cancer cells and facilitates the migration of stroma cells and immune cells to develop more favorable condition for breast cancer.

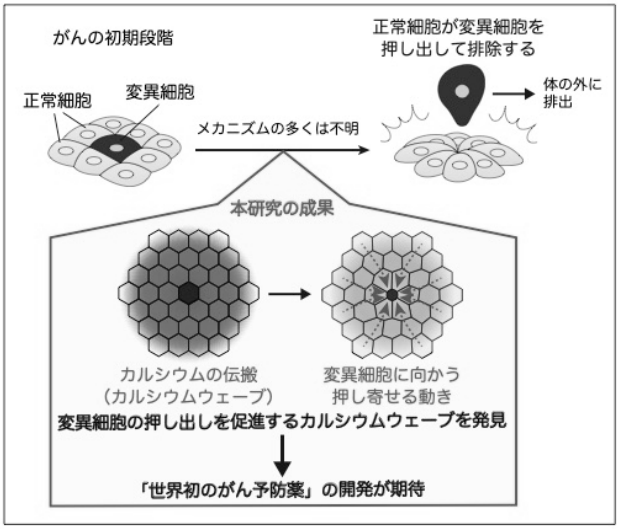


＜2019 年の研究成果，進捗状況及び今後の計画＞

発がんの超初期段階において、変異細胞が正常細胞から排除される際に、変異細胞から周囲の正常細胞に向かってカルシウムイオンが、花火のように同心円状に伝搬することを突き止めた。さらに、このカルシウムウェーブを受けた周囲の正常細胞が、変異細胞に向かって押し寄せるように動くことによって、変異細胞の排除を促進していることが分かりました。変異細胞の排除に伴うカルシウムウェーブは、哺乳類培養細胞層及びゼブラフィッシュの皮膚細胞層の両者で同様に観察されたことから、進化の過程で保存された普遍的な現象であることが示唆されます。これらの研究成果は、

これまでブラックボックスであったがんの超初期段階で生じる現象を世界で初めて明らかにした。本研究によりがん予防薬の開発に繋がることが期待できる。

今後は、がん幹細胞と、その周囲に存在するがん組織を形成する周囲細胞との相互作用に着目した研究を進めていく。がん幹細胞が周囲細胞によってどのように制御されているのか、そのメカニズムを明らかにしたい。



【 研 究 業 績 】

＜発表論文＞

1. Takeuchi Y, Narumi R, Akiyama R, Elisa Vitiello, Shirai T, Nobuyuki Tanimura, Keisuke Kuromiya, Ishikawa S, Kajita M, Tada M, Haraoka Y, Akieda Y, Ishitani T, Fujioka Y, Ohba Y, Yamada S, Hosokawa Y, Toyama Y, Matsui T, Fujita Y. Calcium wave promotes cell extrusion. *Current Biology.* 2020. Feb 24;30(4):670-681.

＜学会発表＞

なし

＜外部資金＞

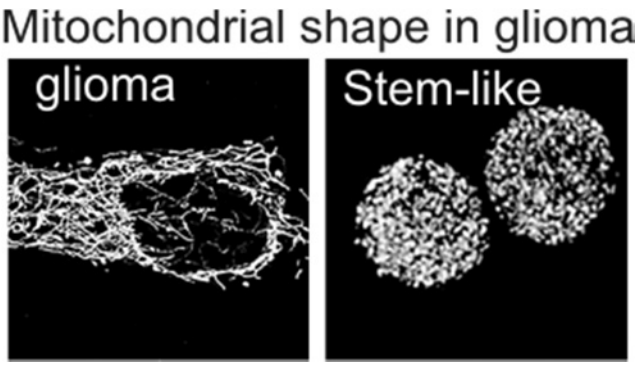
なし

Mitochondrial Dynamics in Stem Cells
ミトコンドリア動態ユニット

Assistant Professor Atsuko KASAHARA 笠原敦子

【Abstract】

Mitochondria play pleiotropic roles in metabolic pathways, calcium and redox homeostasis, and apoptosis. These diverse mitochondrial functions are reflected by their extremely dynamic morphology, and distribution in the cells. Mitochondrial quality, distribution, size, and motility are excellently tuned by their continuous fusion and fission. Equilibrium of fusion and fission shapes the specific mitochondrial network depending on physiological conditions, and cell types. In general, mitochondria appear immature structure with poorly developed cristae in stem cells, while a complex network with developed cristae in differentiated cells. Stem cells are special cell population with self-renewal and differentiation potentials. Healthy stem cells contribute to tissue maintenance and repair, whereas tumour stem-like cells commit tumour malignancy, such as recurrence, drug resistance, and metastasis.
Mitochondrial intracellular localisation in the cell impacts on calcium homeostasis, and Notch1 signalling in embryonic stem cells (Kasahara A. *et al.* Science 2013). Therefore, mitochondria would direct their host cell fate, through controlling signalling cascades by changing their shape and distribution also in cancer cells. We are trying to understand the molecular mechanism of how “mitochondrial dynamics” could control the maintenance and acquisition of stemness in tumour cells.



3D-reconstructed mitochondrial shape in glioma differentiated and stem-like cells (Bossoy E. Y., Kasahara A., *et al*. *EMBO J* 2017)

＜2019 年の研究成果，進捗状況及び今後の計画＞

gefitinib 耐性肺がん細胞についての解析を進め、耐性細胞で、発現が非常に上昇していたミトコンドリア融合因子 Opa1 の発現を低下させると、gefitinib 感受性がやや回復すること、異常なネット状クリステ構造のミトコンドリアの割合もやや減少することを確認した。一方で、小胞体とミトコンドリアとの距離が、幹細胞性に関与している可能性について、人工的にこれら 2 つのオルガネラを近づけるコンストラクトを用いて距離を近づけると、マウス ES 細胞では、多能性マーカーである Oct4 の発現が減少することがわかった。今後は、カルシウム濃度依存的に蛍光する変異 GFP (GCaMP6)を用いて、人工的に 2 つのオルガネラを近づけた細胞の細胞質、ミト

コンドリア、小胞体のカルシウムレベルの挙動の測定などを含めた、Oct4 を減少させるメカニズムや、グリオーマの幹細胞性について調べていく予定である。

【 研 究 業 績 】

＜発表論文＞

なし。

＜学会発表＞

“*Mitochondrial dynamics in malignant progression: retrograde control from mitochondria*”
Kasahara A. The 42th Annual Meeting of the Molecular Biology Society of Japan, 3-6 Dec, Fukuoka, Japan, Workshop AMED-CREST/PRIME [Mechanobiology] “Novel linkage between the structure and molecular function in mitochondria” Oral presentation

＜外部資金＞

科学研究費助成事業（学術研究助成基金助成金）基盤研究（C）
「がん幹細胞性におけるミトコンドリア動態の果たす役割を明らかにする」
3,300千円　3年間

第33回北國がん基金
「がん分子標的薬剤耐性機構におけるミトコンドリア動態・機能の役割」
1,000千円

Cancer–Immune System Interactions
がん-免疫系相互作用ユニット

Assistant Professor　　Kohsuke Tsuchiya 土屋 晃介

【 Abstract 】

Caspase-1 is activated in response to various inflammatory stimuli, including microbial pathogens, endogenous danger signals, and irritants. Once activated, caspase-1 induces pyroptosis, a form of regulated necrosis, characterized by cell membrane disruption and release of cellular contents, leading to inflammation. Gasdermin D (GSDMD), a caspase-1 substrate, mediates pyroptosis: after being cleaved by caspase-1, the N-terminal fragment of GSDMD forms pores on the plasma membrane, resulting in water influx, cell swelling, and membrane rupture. Recently, we found that apoptosis is induced after caspase-1 activation in GSDMD-deficient cells, suggesting that caspase-1 can initiate both pyroptosis and apoptosis, depending on the expression of GSDMD. We previously found that in the absence of GSDMD, caspase-1 processes Bid into the mature form tBid, leading to apoptosis via the mitochondrial pathway. However, an additional mechanism that mediates this cell death independently of Bid has also been suggested. Here, we investigate the mechanism and physiological significance of caspase-1-induced cell death. Our study suggests that caspase-7, a substrate of caspase-1, mediates apoptosis induced by caspase-1 in a Bid-independent manner. It has also been suggested that caspase-1-induced apoptosis can occur in neurons and mast cells that express undetectable or low levels of GSDMD, respectively. Moreover, release of IL-1β and maturation of IL-1α were dependent on cell death (pyroptosis or apoptosis) following inflammasome activation. These results suggest that caspase-1 induces cell death in various cell types through multiple signal transduction pathways and that caspase-1-induced cell death plays a critical role in IL-1-mediated inflammation.

＜2019 年の研究成果，進捗状況及び今後の計画＞

Caspase-1 による細胞死誘導の機序および意義について詳細な検討を行った。前年までに caspase-1 が Bid の切断を介してアポトーシスを誘導することを明らかにしていたが、caspase-1 によるアポトーシス誘導のシグナル伝達分子として新たに caspase-7 を見出した。また、神経細胞やマスト細胞のような GSDMD 発現レベルが低い細胞種において実際に caspase-1 依存的なアポトーシスが起きうることを示した。さらに、caspase-1 による細胞死誘導が IL-1βの放出や IL-1αの成熟化に重要な役割を果たすことを明らかにした。今後、caspase-1 誘導性細胞死の生理的意義についてさらに検討を進め、炎症性疾患の病態形成やがん微小環境などにおける役割を明らかにする。

【 研 究 業 績 】

＜発表論文＞

原著（研究室主体）

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2. Fang R, Uchiyama R, Sakai S, Hara H, Tsutsui H, Suda T, Mitsuyama M, Kawamura I, Tsuchiya K. ASC and NLRP3 maintain innate immune homeostasis in the airway through an inflammasome-independent mechanism. Mucosal Immunol. 2019 12:1092-1103.
3. Mahib MR, Hosojima S, Kushiyama H, Kinoshita T, Shiroishi T, Suda T, Tsuchiya K. Caspase-7 mediates caspase-1-induced apoptosis independently of Bid. Microbiol Immunol. 2019 doi: 10.1111/1348-0421.12756.

＜学会発表＞

1. Kohsuke Tsuchiya, Mamunur Rashid Mahib, Takashi Suda. Caspase-1 initiates apoptosis in the absence of gasdermin D. The 17th International Congress of Immunology (IUIS2019). Oct 22, Beijing, China
2. Kohsuke Tsuchiya, Takashi Suda. Pyroptosis enhances antibiotic therapy of listeriosis. 第 92 回日本細菌学会総会 4 月 25 日 札幌市
3. Kohsuke Tsuchiya, Takashi Suda. Gasdermin D (GSDMD) mediates IL-1α maturation during inflammasome formation. 第 48 回日本免疫学会学術集会 12 月 12 日 浜松市

＜外部資金＞

1. 土屋晃介（研究代表者）令和元年度 科研費 基盤研究（C)「細菌感染治療の分子基盤を自然免疫機構と化学療法の協調的相互作用から理解する試み」 1,430 千円 (直接経費 : 1,100 千円、間接経費 : 330 千円)
2. 土屋晃介 武田科学振興財団 医学系研究助成（基礎)「カスパーゼ-1 による細胞死誘導の分子機序とインフラマソーム関連疾患における役割」2,000 千円
3. 土屋晃介　琉球大学熱帯生物圏研究センター・2019 年度 共同利用共同研究事業「肺胞上皮におけるインターロイキン-17F 産生の意義と分子基盤」 250 千円

＜その他＞

金沢大学プレスリリース

https://www.kanazawa-u.ac.jp/rd/67529
https://www.kanazawa-u.ac.jp/rd/72580

西南大学プレスリリース（中国語）

http://222.198.125.159/seeyon/xndxNewsData.do?method=userView&id=R3YxdlVxU1FBRytQL3UrcFNoakR6MXVETzNHN0hMUEY=&t=1562829499651