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

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研究課題名（和文）Developing mouse models of inflammation-driven invasive gastric cancer to reveal novel therapeutic targets

研究課題名（英文）Developing mouse models of inflammation-driven invasive gastric cancer to reveal novel therapeutic targets

研究代表者
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研究成果の概要（和文）：新たな治療標的となるがん幹細胞集団を見出し、胃がん進行に対するそれらの寄与を見積もるためには、ヒト胃がんの発生を正確に模倣できる侵襲性・転移性胃がんマウスモデルの開発が喫緊の課題である。我々は、Lgr5陽性の胃がん細胞ががん幹細胞である可能性を検証し、胃がんの進展に対するそれらの役割を明らかにするため、炎症依存的に侵襲性胃がんを発症する新規胃がんマウスモデルを作製した。これらのマウスモデルは、将来の抗がん治療の有効性/選択性を正確に評価するためのスクリーニング方法としても非常に重要であり、得られた知見は独創的で胃がん研究分野に大きな影響を与えると予想される。

研究成果の学術的意義や社会的意義
胃がんの発生機序に対する私たち理解は限られており、このことが効果的な治療法の開発を妨げている。我々は、炎症を伴って発生するヒト胃がんを模倣する新たな胃がんマウスモデルを樹立し、生体内・生体外の解析を通してLgr5遺伝子を発現する胃がん細胞が、がん幹細胞である可能性を示唆する結果を得た。また、胃がんの悪性化に寄与する新たなドライバー変異を持つマウスモデルを作製し、それらの遺伝子変異が胃がんの進展に与える影響を解析した。これらの研究手法や得られた結果は、胃がんの進行を制御するメカニズムの迅速な解明や胃がんに対する新たな治療法の開発につながることが期待される。

研究成果の概要（英文）：There is an urgent, unmet need for accurate mouse models of invasive, metastatic gastric cancer that can be used to derive important mechanistic insight into gastric cancer progression and to identify candidate cancer stem cell populations as novel therapeutic targets. We generated the first inflammation-driven mouse models of invasive gastric cancer for use in studying gastric cancer development in the stomach, with a particular focus on understanding the role of Lgr5+ stem cells and Lgr5+ cancer stem cells in this process.
These mouse models will also be invaluable as screening modalities for accurately evaluating the efficacy/selectivity of future anti-cancer therapeutics. The mouse models and experimental procedures detailed here are original and will deliver results that are expected to have a major impact on the gastric cancer research field.

研究分野：幹細胞生物学

キーワード：Gastric cancer　Mouse model　Inflammation　Lgr5　Organoid

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Gastric cancer is a complex disease that often arises in a setting of chronic inflammation. Despite recent consortia led efforts to molecularly classify gastric cancers to try and stratify treatment regimens according to underlying mutational spectra, gastric cancer remains a relatively poorly understood disease with a poor prognosis for most patients. In particular, a lack of accurate mouse models of invasive, metastatic human gastric cancer has severely hampered gastric cancer research efforts and the development of more effective, targeted therapeutics for clinical use.

My Singapore research group is focused on evaluating a potential cancer stem cell function for the Lgr5+ cells present within inflammation-independent gastric tumours using in lineage tracing and in vivo ablation strategies. In Kanazawa, I would like to leverage on the extensive knowledge and mouse models available through my collaborator, Professor Masanobu Oshima at the Cancer Research Institute to study the effects of chronic inflammation on stem cell-driven cancer formation and progression in the corpus stomach. This is physiologically relevant because the majority of human gastric cancer is considered to arise in a setting of chronic inflammation caused by infection with *Helicobacter Pylori*.

２．研究の目的

Aim1: Investigating the contribution of corpus Lgr5+ stem cells to inflammation-driven gastric cancer in vivo:

We propose to generate a compound mouse model that will facilitate: i) a direct evaluation of Lgr5+ gastric stem cell behaviour in a chronic Wnt-driven inflammation setting via *in vivo* lineage tracing or *ex vivo* culture assay ii) Characterization of inflammation-driven changes to Lgr5+ stem cell population size, proliferation status & transcriptome iii) *In vivo* ablation of Lgr5+ stem cells to evaluate their contribution to inflammation-induced gastric cancer formation. These in vivo assays are expected to deliver invaluable new mechanistic insight into inflammation-driven gastric cancer formation in the corpus. Identification of Lgr5+ corpus cells as key drivers of gastric cancer initiation and progression is expected to reveal novel opportunities for therapeutic intervention in the clinic.

Aim2: To generate the first inflammation-driven mouse models of invasive, metastatic gastric cancer to facilitate a functional evaluation of Lgr5-expressing tumour cells as cancer stem cells:

Recently, we have identified Lgr5-expressing chief cells in the corpus stomach, which serve as reserve stem cells to effect epithelial renewal following oxyntic atrophy (Leushacke M. *et al.*, ***Nature Cell Biology***, 2017). These reserve stem cells drive the Spasmolytic Polypeptide-Expressing Metaplasia (SPEM) in the stomach following conditional mutation, highlighting their likely contribution to gastric cancer initiation *in vivo*. Although, we currently do not know whether those Lgr5-expressing cells function as cancer stem cells. We, therefore, propose to employ our inflammation-driven invasive gastric cancer model to functionally evaluate tumour-resident Lgr5-expressing cells at different stages of the carcinogenic process as candidate cancer stem cells using *in vivo* ablation, lineage tracing and *ex vivo* culture/transplantation assays. This is expected to reveal novel therapeutic targets mediating selective elimination of these cancer stem cells in the clinic.

Aim3: To evaluate the contribution of candidate TCGA derived driver mutations such as RhoA and RNF43 to in vivo gastric cancer formation:

Human TCGA analyses have identified novel candidate driver mutations of gastric cancer, including activating *RhoA* and inactivating *RNF43* mutations, but their role in driving gastric cancer is currently unknown. We will generate conditional *RhoA* & *RNF43* alleles and subsequently employ the Cldn18-ires-CreERT2 driver to introduce these mutations into the gastric epithelium of Gan [WNT/COX- 2/PGE2] mice, which exclusively develop non-invasive inflammation-driven gastric adenomas in the corpus region after 6 months. Any observed changes to the rate of tumour formation or progression towards more invasive, malignant disease following introduction of the *RhoA*/*RNF43* mutations would validate their identity as gastric cancer drivers and identify them as potential therapeutic targets. Any observed changes to the rate of tumour formation or progression towards more invasive, malignant disease following introduction of the *RhoA*/*RNF43* mutations would validate their identity as gastric cancer drivers and identify them as potential therapeutic targets.

３．研究の方法

This 3-year research plan will involve the generation of various mouse models that facilitate a rigorous evaluation of the contribution of Lgr5-expressing stem cells and putative Lgr5-expressing cancer stem cells to gastric cancer formation and progression. We will also generate new conditional mouse models to evaluate the contribution of novel driver mutations to gastric cancer progression in vivo. The first year will be spent generating the various compound mutant mouse lines needed to conduct the various research aims. In the second and third years, we will perform in vivo lineage tracing, ex vivo culture analyses, cancer modeling, transplantation, expression profiling and in vivo ablation studies to provide mechanistic insight into gastric cancer progression and to reveal novel therapeutic targets.

Aim1: Investigating the contribution of corpus Lgr5+ stem cells to inflammation-driven gastric cancer in vivo:

We generate compound mouse models in combination with Lgr5 reporter mice (Lgr5DTR-EGFP or Lgr5-2A-CreERT2/RosatdTomato) and Gan mutations. In the Gan/Lgr5-DTR-EGFP mouse model, Lgr5+ corpus stem cells express both EGFP reporter gene and the diphtheria toxin receptor (DTR). Expression of EGFP facilitates the visualization and isolation of Lgr5+ corpus stem cells, whilst the DTR expression facilitates the selective *in vivo* ablation of the Lgr5+ corpus stem cells via administration of diphtheria toxin. In the Gan/Lgr5-2A-CreERT2/RosatdTomato mouse model, Tamoxifen administration activates tdTomato reporter gene expression in the Lgr5+ corpus cells, facilitating an evaluation of Lgr5+ stem cell activity in an inflammatory setting via *in vivo* lineage tracing.

Aim2: To generate the first inflammation-driven mouse models of invasive, metastatic gastric cancer to facilitate a functional evaluation of Lgr5-expressing tumour cells as cancer stem cells:

Using our new gastric Cre driver, Cldn18-ires-CreERT2, we have generated invasive models of gastric cancer. However, these mouse models do not incorporate chronic inflammation, as commonly found in human gastric cancers. We, therefore, aim to generate 3 independent inflammation-driven mouse models of gastric cancer:

Model 1: Cldn18-ires-CreERT2/Gan/Ptenhom/KrasG12Dhet/P53hom/Lgr5-DTR-EGFP
Model 2: Cldn18-ires-CreERT2/Gan/TGFbRIIhom//P53mut/Lgr5-DTR-EGFP
Model 3: Cldn18-ires-CreERT2/Apchom/C2ME/Ptenhom/KrasG12Dhet/P53hom/Lgr5-DTR-EGFP

Using those mouse models, we will evaluate the cancer stem cell identity of tumour-resident Lgr5+ cells via *in vivo* ablation.

Aim3: To evaluate the contribution of candidate TCGA derived driver mutations such as RhoA and RNF43 to in vivo gastric cancer formation:

Activating *RhoA* and loss-off-function *RNF43* mutations have been identified as potentially novel driver mutations in human gastric cancer. Therefore, we functionally evaluate the role of these mutations in gastric cancer progression by generating the corresponding conditional mouse mutants using either existing ES cells (IMSR) or via conventional gene targeting/Crispr-Cas9 methodologies. We will then incorporate these mutations into our Cldn18-CreERT2 driven conditional cancer models (see below):

Model 1: Cldn18-ires-CreERT2/Gan/RhoAhet
Model 2: Cldn18-ires-CreERT2/APChom/RhoAhet
Model 3: Cldn18-ires-CreERT2/Gan/RNF43hom
Model 4: Cldn18-ires-CreERT2/APCflox/flox/RNF43hom
Model 5: Cldn18-ires-CreERT2/Ptenhom/P53hom/Krashet/RhoAhet
Model 6: Cldn18-ires-CreERT2/Ptenhom/P53hom/Krashet/RNF43hom

Models 1-4 will facilitate an evaluation of a potential role for *RhoA*/*RNF43* mutations in driving progression from benign polyps to more advanced gastric cancers.

Models 5-6 will facilitate an evaluation of a potential role for *RhoA*/*RNF43* mutations in accelerating cancer development, invasion and/or metastasis.

Activation of the various mutations will be achieved by administering tamoxifen to adult conditional mutation mice, followed by evaluation of cancer progress via histological/marker analyses over the course of the next 6 months. Successful activation of *RhoA* mutations will be confirmed by IHC for active *RhoA*. Successful loss of *RNF43* function will be confirmed by IHC/in-situ hybridization for *RNF43*.

In parallel, we will isolate tumour tissue from the various models for generating epithelial organoids *ex vivo* to determine any phenotypic changes to their growth characteristics as compared to non-cancer organoids.

４．研究成果

There is an urgent, unmet need for accurate mouse models of invasive, metastatic gastric cancer that can be used to derive important mechanistic insight into gastric cancer progression and to identify candidate cancer stem cell populations as novel therapeutic targets. The purpose of the research was to generate the first inflammation-driven mouse models of invasive gastric cancer for use in studying gastric cancer development in the stomach, with a particular focus on understanding the role of Lgr5+ stem cells and Lgr5+ cancer stem cells in this process.

Aim1: Investigating the contribution of corpus Lgr5+ stem cells to inflammation-driven gastric cancer in vivo:

We generated Gan/Lgr5-DTR-EGFP mice and Gan/Lgr5-2A-CreERT2/RosatdTomato mice. Using those models, we found Lgr5+ tumour cells reside in Gan polyps. Those cells expressed gastrointestinal stem cell markers (e.g. Lgr5, Troy) while did not express differentiation markers (e.g. Muc5ac). Furthermore, ablation of Lgr5+ tumour cells by the addition of diphtheria toxin (DT) significantly reduced the tumour mass and suppressed metastasis suggesting that Lgr5+ cells are potential cancer-initiating cells in inflammation-dependent gastric tumours. While after discontinuation of the DT treatment, Lgr5+ tumour cells re-appeared and tumours started to grow again suggesting that Lgr5+ tumour-initiating cells can arise from Lgr5- tumour cells.

Aim2: To generate the first inflammation-driven mouse models of invasive, metastatic gastric cancer to facilitate a functional evaluation of Lgr5-expressing tumour cells as cancer stem cells:

We introduced the Cldn18-ires-CreERT2 strain and tried to establish new gastric cancer mouse models. The schedule was significantly delayed because the breeding did not go well. Finally, we have succeeded to establish new models and those strains are under-investigation at this moment.

Aim3: To evaluate the contribution of candidate TCGA derived driver mutations such as RhoA and RNF43 to in vivo gastric cancer formation:

We generated conditional mouse mutants which have LSL-mutant RhoA alleles or Floxed Rnf43 alleles. Successful activation of RhoA mutations was confirmed by IHC and successful loss of RNF43 function was confirmed by IHC/in-situ hybridization for RNF43.

Then, we incorporated these mutations to Cldn18-ires-CreERT2/Gan or Cldn18-ires-CreERT2/APCflox/flox or Cldn18-ires-CreERT2/Ptenhom/P53hom/Krashet to generate new gastric cancer mouse models. We have succeeded to establish mouse models and activation of the various mutations was achieved by administering tamoxifen to adult conditional mutation mice, followed by evaluation of cancer progress via histological/marker analyses over the course of the next 6 months. Those mice have been followed-up at this moment.

Furthermore, we have isolated tumour tissue from the various models and generated epithelial organoids ex vivo to determine any phenotypic changes to their growth characteristics as compared to non-cancer organoids.

These mouse models will also be invaluable as screening modalities for accurately evaluating the efficacy/selectivity of future anti-cancer therapeutics. The mouse models and experimental procedures detailed here are original and will deliver results that are expected to have a major impact on the gastric cancer research field.

５．主な発表論文等

〔雑誌論文〕　計13件（うち査読付論文　13件／うち国際共著　5件／うちオープンアクセス　1件）

１．著者名 Tan Si Hui et al.	４．巻 578
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３．雑誌名 Nature	６．最初と最後の頁 437～443
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41586-020-1973-x	査読の有無 有
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 該当する

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3．書名 Progress in Molecular Biology and Translational Science	

〔産業財産権〕

〔その他〕

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6．研究組織

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６．研究組織（つづき）

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