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　３ 年　 ６ 月 １４ 日現在

機関番号：１１３０１
研究種目：若手研究
研究期間：2019～2020
課題番号：１９Ｋ１６５１２

研究課題名（和文）Development of an Nrf2 inhibitor for the treatment of Nrf2-addicted cancer

研究課題名（英文）Development of an Nrf2 inhibitor for the treatment of Nrf2-addicted cancer

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：I developed a novel assay to identify compounds which could selectively kill tumour cells with high levels of NRF2. Thus, I identified two different classes of compounds, geldanamycin-derived HSP90 inhibitors, and the DNA damaging agent mitomycin C, which both displayed NRF2-dependent toxicity.

研究成果の学術的意義や社会的意義
As there are no approved drugs which can be used to treat patients with NRF2-dependent tumours, there is an urgent unmet clinical need to identify such drugs. In this project, I identified the chemotherapy drug mitomycin C to be an ideal candidate for drug repositioning to NRF2-dependent tumours.

研究成果の概要（英文）：Activation of the KEAP-NRF2 pathway is observed in approximately 30% of human lung tumours, where it is associated with a poor prognosis and reduced overall survival for patients. Despite the fact that NRF2 is a validated driver of aggressive cancer growth, the complete lack of approved drugs which can target oncogenic NRF2 signaling means that there is an urgent clinical need to identify compounds which display efficacy against NRF2-dependent tumours. In this project, I developed a novel assay to identify compounds which could selectively kill tumour cells with high levels of NRF2 activity. Using this approach, I identified two different classes of compounds, geldanamycin-derived HSP90 inhibitors, and the DNA damaging agent mitomycin C, which both displayed NRF2-dependent toxicity.

研究分野：Medical Biochemistry

キーワード：Keap1-Nrf2

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Pan-cancer genomic analyses have identified a multitude of signaling pathways which drive and sustain tumorigenesis. Unfortunately, in many cases the therapeutic exploitation of these validated cancer targets is limited by the lack of efficacious drugs which can specifically inhibit oncogenic signaling. For example, many tumour suppressors and oncogenic transcription factors lack deep, druggable binding pockets which makes the pharmaceutical manipulation of their activities particularly challenging. This inability to chemically modulate these bona fide cancer drivers presents a significant bottleneck in the fight against cancer.

One important oncogenic pathway whose activity cannot currently be inhibited by therapeutic interventions is the KEAP1-NRF2 pathway. Activating mutations in KEAP1-NRF2 signaling are present in 34% of squamous cell lung carcinoma, 22% of lung adenocarcinoma, 30% of oesophageal carcinoma, and 19% of hepatocellular carcinoma, and are associated with a poor prognosis and low overall patient survival.

２．研究の目的

Current strategies for targeting NRF2-dependent tumours are primarily focused on the development of direct NRF2 inhibitors. These first-generation NRF2 inhibitors, including brusatol and halofuginone, are general protein translation inhibitors which do not exhibit specificity for the KEAP1-NRF2 pathway, and therefore have limited clinical potential. As it is particularly challenging to develop drugs which directly inhibit the function of transcription factors like NRF2, we pursued a synthetic lethal strategy to specifically target NRF2-dependent tumours, as this obviates the need to identify direct NRF2 inhibitors. Because NRF2 regulates the expression of many drug metabolizing enzymes, it is an excellent candidate for synthetic lethal screening as its target genes may metabolize and activate prodrugs specifically in tumour cells with aberrant NRF2 activation. In the context of NRF2-dependent cancer, a synthetic lethal compound would only exhibit toxicity in cells with high levels of NRF2 activity, leaving the wild-type cells within the patient relatively insensitive to any harmful effects. This strategy provides a large therapeutic window for treatment, and allows for the development of compounds to target pathways often considered “non-druggable”.

３．研究の方法

In order to identify compounds which are synthetic lethal with high levels of NRF2 activity, we developed a phenotypic screen based on an isogenic pair of fluorescently labelled Hepa1 cells. CRISPR-Cas9 was used to knockout Keap1, resulting in the constitutive activation of Nrf2 and the upregulation of Nrf2-dependent target gene expression. Stable clones of the parental wild-type Hepa1 cells expressing EGFP, and

Keap1 KO cells expressing mCherry, were generated, which allowed the genetic identity of the cells to be tracked throughout the screening process due to their differential fluorophore expression. This enabled us to the mix the WT-GFP and Keap1 KO-mCherry cells in the same microplate wells during the screen, guaranteeing that the cells experienced the same conditions when treated with the library of screening compounds, while simultaneously generating a greater dynamic range for the screening assay. Using this approach, any compound which could significantly decrease the ratio of mCherry:GFP would be considered a synthetic lethal hit (Fig 1).

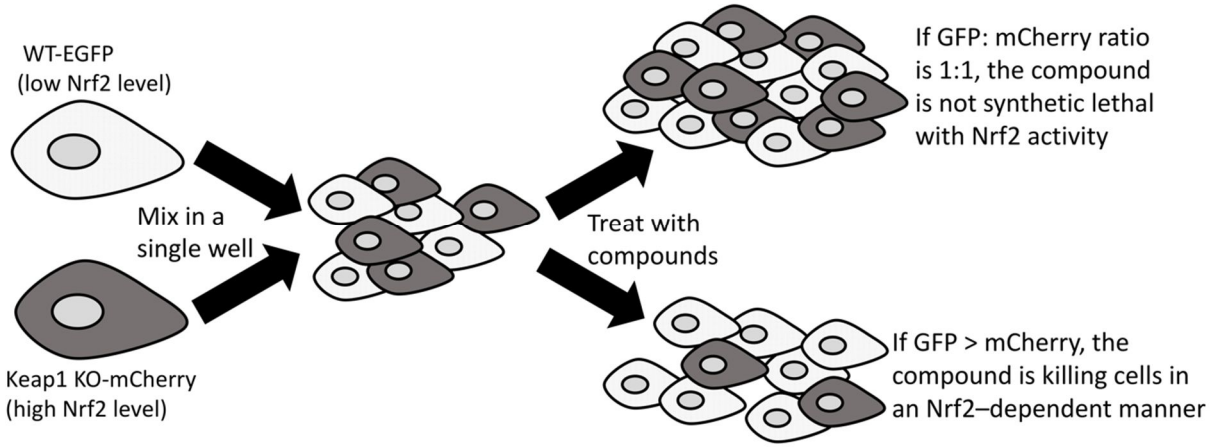

FIG 1. The novel fluorescence-based screening system which I used to identify NRF2-dependent synthetic lethal compounds.

４．研究成果

In this study, we developed a novel synthetic lethal screening strategy to identify compounds which specifically kill cells with high levels of NRF2 activity. Through this approach, we identified two different classes of compounds which show enhanced toxicity towards cells with activated NRF2 signaling.

The first class consists of three compounds that are based on the geldanamycin scaffold, and function as HSP90 inhibitors. Mechanistically, we show that NRF2 target genes effectively turn the geldanamycin-derived compounds into prodrugs which are selectively metabolized into more potent HSP90 inhibitors in cells with aberrant NRF2 activity. Together, our findings demonstrate that geldanamycin-based compounds represent excellent candidates for drug repositioning to target the currently undruggable KEAP1 and NRF2 mutations in human cancer.

Furthermore, we identified the DNA alkylating agent mitomycin C to also be a compound which displays enhanced toxicity in cells with activated NRF2 signaling. Mechanistically, we found that a number of NRF2 target genes, including CYPOR, NQO1 and enzymes in the pentose phosphate pathway, are all required for the intracellular bioactivation of mitomycin C. Therefore, enhanced NRF2 activity results in the increased bioactivation of mitomycin C, and therefore enhanced toxicity in cells with activated NRF2 signaling. As mitomycin C is already approved for clinical use, we believe that it represents an excellent candidate for drug repositioning to target the currently

untreatable aberrant NRF2 activation in human tumours.

5．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　0件／うちオープンアクセス　0件）

1．著者名 Baird Liam、Yamamoto Masayuki	4．巻 41
2．論文標題 NRF2-Dependent Bioactivation of Mitomycin C as a Novel Strategy To Target KEAP1-NRF2 Pathway Activation in Human Cancer	5．発行年 2021年
3．雑誌名 Molecular and Cellular Biology	6．最初と最後の頁 1-18
掲載論文のDOI（デジタルオブジェクト識別子） 10.1128/MCB.00473-20	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 －

1．著者名 Baird Liam、Suzuki Takafumi、Takahashi Yushi、Hishinuma Eiji、Saigusa Daisuke、Yamamoto Masayuki	4．巻 40
2．論文標題 Geldanamycin-Derived HSP90 Inhibitors Are Synthetic Lethal with NRF2	5．発行年 2020年
3．雑誌名 Molecular and Cellular Biology	6．最初と最後の頁 1-22
掲載論文のDOI（デジタルオブジェクト識別子） 10.1128/MCB.00377-20	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 －

1．著者名 Baird Liam、Yamamoto Masayuki	4．巻 40
2．論文標題 The Molecular Mechanisms Regulating the KEAP1-NRF2 Pathway	5．発行年 2020年
3．雑誌名 Molecular and Cellular Biology	6．最初と最後の頁 1-23
掲載論文のDOI（デジタルオブジェクト識別子） 10.1128/MCB.00099-20	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 －

〔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