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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）Hotspot Synonymous Cancer Mutations Part 1: Effect on Cap-independent Translation of New HRAS Isoform

研究課題名（英文）Hotspot Synonymous Cancer Mutations Part 1: Effect on Cap-independent Translation of New HRAS Isoform

研究代表者
Ｃａｎｄｅｉａｓ　Ｍａｒｃｏ（Candeias, Marco）

京都大学・医学研究科・講師

研究者番号：５０７５０５８５

交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：Ras genes are the most mutated proto-oncogenes in cancer. Here we identified a new Ras protein: p14HRas. We identified the mechanisms of regulation of p14 and propose a new model for oncogenicity.

研究成果の学術的意義や社会的意義
Ras genes are the most mutated proto-oncogenes in cancer. We propose a new mechanism for oncogenicity in cancer involving a new HRas proto-oncogene. Further studies can be aimed at targeting p14HRas or its mechanisms of expression (also identified here) for a new therapy to treat cancer patients.

研究成果の概要（英文）：Ras genes are the most mutated proto-oncogenes in cancer. Here we identified a new Ras protein: p14HRas. We identified the mechanisms of regulation of p14 and investigated p14’s mutation and upregulation in cancer. We discovered the mechanism of action. We propose a new mechanism for oncogenicity in cancer involving a new proto-oncogene (p14HRas) and an alternative translation mechanism. Further studies can be aimed at targeting p14HRas or its mechanisms of expression for therapy in cancer patients.

研究分野：cancer

キーワード：cancer　HRas　HRas mRNA　HRas isoform　HRas translation　cancer mutation　p14HRas　bladder cancer

１．研究開始当初の背景

HRAS encodes the H-Ras protein that belongs to the Ras family of small GTP-binding proteins alongside K-Ras and N-Ras. Ras proteins regulate cell division via the MAPK/ERK pathway in response to growth factor stimulation. HRAS is a proto-oncogene commonly mutated in cancer, but some mutational hotspots in HRAS remain to be investigated. Well-studied mutational hotspots in HRAS are G12, G13 and Q61. Missense mutations in these codons favor binding of Ras to GTP, leading to Ras activation, cell division and carcinogenesis (Prior et al. Cancer Res. 2012). With this project we set to study the 4th most common mutation in HRAS in cancer: mut4. Mut4 in HRAS is associated with cancer risk but the underlying mechanisms are not known.

Internal ribosome entry sites (IRESs) are mRNA structures that allow for translation to be initiated internally in the middle of the mRNA. IRESs are regulated by distinct translation factors and do not require assistance from several components of the canonical translation machinery such as the cap-binding factor eIF4E (Holcik et al. Nat Rev Mol Cell Biol 2005). I have established methods for identifying mRNA regions containing IRESs and I have identified 3 different IRESs already: 1. p53 IRES(40) (Candeias et al. Oncogene 2006), 2. mTOR IRES (Marques-Ramos et al. RNA 2017) and 3. p53 IRES(160) (Lopez-Iniesta et al. bioRxiv 2023). The first is present in the coding region of p53 mRNA and induces D40p53 isoform expression during stress of the endoplasmic reticulum causing cell-cycle arrest in the G2-phase (my previous work, Bourougaa et al. Mol Cell 2010).

２．研究の目的

The purpose of the study was to:

A. Elucidate the presence of mut4 in cancer;
B. Identify and characterize a new IRES in the HRAS mRNA;
C. Identify and initiate the characterization of a new H-Ras protein isoform;
D. Identify new cancer-associated mutations in HRAS and other genes using bioinformatics.

３．研究の方法

Methods used consisted in mapping the IRES; Dual-luciferase reporter assays; drug treatments; bicistronic assays using WB; mutational analyses; sequencing and genotyping of bladder cancer cell lines and bladder cancer samples from patients.

４．研究成果

With this project, we could make significant advances in Ras research. We identified a new Ras protein, that we termed p14HRas; we identified the mechanisms of regulation of p14 (Internal Ribosome Entry Site [IRES]-mediated translation); we characterized the function of this new isoform and observed that it induces cell proliferation; we calculated the incidence of mut4 in bladder cancer (~30%); mut4 also affected cell proliferation but did not seem to affect p14 levels or function. These findings propose a new mechanism for oncogenicity in cancer, involving a new proto-oncogene (p14HRas) and a new alternative translation mechanism. Further studies can be aimed at targeting p14HRas and HRas-IRES for therapy in cancer patients.

５．主な発表論文等

（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　４　件）

① Candeias MM, Hagiwara M & Matsuda M. EMBO Rep. 17(11):1542-1551, Nov, 2016 https://doi.org/10.15252/embr.201541956

② Tamura N & Candeias MM 実験医学, Vol.35 No.14, Sep, 2017 https://www.yodosha.co.jp/yodobook/book/9784758101677/ / ISBN 978-4-7581-0167-7

③ Marques-Ramos A, Candeias MM, Menezes J, Lacerda R, Willcocks M, Teixeira A, Locker N & Romao L RNA rna.063040.117, Aug, 2017 http://rnajournal.cshlp.org/content/23/11/1712.short/ doi:10.1261/rna.063040.117

④ Lacerda R, Menezes J, Candeias MM. Alternative Mechanisms of mRNA Translation Initiation in Cellular Stress Response and Cancer. Adv Exp Med Biol. 2019;1157:117-132. doi: 10.1007/978-3-030-19966-1_6. PMID: 31342440.

〔学会発表〕(計　5　件)

〔図書〕(計　　件)

〔産業財産権〕

○出願状況（計　　件）

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〔その他〕
ホームページ等
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６．研究組織
(1)研究代表者
Marco Candeias
Molecular and RNA Cancer Unit, Graduate School of Medicine Bldg. E Rm 202, Kyoto University
研究者番号: 50750585

(2)研究分担者

(3)連携研究者

(4)研究協力者
(3)