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| 論文題目 | Conserved Double Translation Initiation Site for Δ160p53 Protein Hints at Isoform's Key Role in Mammalian Physiology<br>（哺乳類間で保存されたp53タンパク質の二つ翻訳開始点はΔ160p53アイソフォームの重要な生理学的役割を示唆する） | | |

(Dissertation Summary)

p53 is a tumor suppressor gene that was discovered over 40 years ago and has been widely studied since then. It is a complex gene with more than one promoter, and it can express several protein isoforms through different translation initiation sites or alternative splicing. p53 has been found to be one of the most mutated genes in human cancers. Two of the main reasons that explain this fact are, on one hand, that its longer protein isoforms protect against carcinogenesis, whereas on the other hand its short C-terminal protein isoforms favor cancer progression.

One of the latest p53 isoforms discovered is Δ160p53, which promotes invasion and survival of cells, and is translated from AUG160 codon. There is another AUG (AUG169) 9 codons downstream of the translation initiation site for Δ160p53. In this study it is shown by toeprinting assay that codon AUG169 can be recognized by the translation machinery. Indeed, Western blot analyses of several cell lines, especially after exposure to DNA damage or endoplasmic reticulum (ER) stress, showed the presence of two protein bands around the expected size of Δ160p53. It was later confirmed that a protein similar to Δ160p53, Δ169p53, is in fact translated from AUG169. When AUG160 is mutated, Δ169p53 protein expression levels get to similar levels to that of Δ160p53. Both Δ160p53 and Δ169p53 could also be translated when a frameshift mutation was introduced upstream of AUG160, verifying that they are translation products and excluding the possibility of them being a cleavage product from a bigger p53 protein isoform.

When the translation dynamics were checked through a dual luciferase reporter system, it was found that under mild stress conditions the presence of either of the two translation initiation sites was sufficient for high protein expression. Conversely, under normal culture conditions, the absence of just one of them was sufficient to induce a large drop in translation. These findings suggest a redundancy in the translation initiation mechanism during stress response, which is not seen in normal growth conditions.

When the function of Δ169p53 protein was assessed, it was observed by MTT assay that Δ169p53 promotes cell survival under mild DNA damage conditions as efficiently as Δ160p53 protein isoform. It was also shown that both isoforms shared the same phenotype as well in the case of mild stress induced by oxidative damage as in this instance they both failed to counteract the toxic effect.

The fact that the translation and pro-survival activity of Δ169p53 are enhanced in the presence of stress conditions is in line with the idea that it might enhance cell fitness and invasiveness in a similar way to Δ160p53, in line with previous studies showing that these stress conditions mimic an oncogenic cell state.

By sequence comparison analysis it is shown that for chordate species with an orthologue to human AUG160 (all mammal species investigated), most of them possess an ortholog to human AUG169 as well. On the contrary, none of the chordate species that lack ortholog AUG160 possessed AUG169, even when that RNA of the p53 gene was well conserved.

It can be inferred by the results stated that Δ169p53 might not be a different and independent protein isoform, but instead that the double translation initiation site reinforces the translation of both proteins, which have a critical role in cell homeostasis. This protein expression mechanism seen here in mammals could hint at a more general mechanism in the evolution path of mammals to ensure the expression of essential proteins in cell homeostasis.

（論文審査の結果の要旨）

*p53* はヒトのがんで最も高頻度で変異が認められる遺伝子である。また、異なる翻訳開始部位によって複数のアイソフォームが存在していることが知られている。これまでに、p53タンパク質のアイソフォームの一つ（Δ160p53）が、がん細胞の生存と浸潤を促進することが明らかになっている。しかし、その他の翻訳開始部位を有するアイソフォームについてはまだ分かっていないことが多く残されている。

本研究では、Δ160p53の翻訳開始コドンとして知られているコドン160の9コドン下流に、翻訳機構によって認識される翻訳開始可能部位があることを見出した。AUG160の変異によって翻訳が開始されない時、代わりにAUG169から翻訳が開始され、DNA損傷後にΔ160p53と同程度の効率で細胞生存を促進するΔ169p53が産生された。興味深いことに、ヒトのAUG160に対するオルソログを持つほとんどすべての哺乳類種は、AUG169に対するオルソログも持っている。一方、*p53*遺伝子にAUG160が存在しない非哺乳類種はAUG169を有しない。今回の結果から、これら2箇所の翻訳開始部位が、細胞の恒常性維持に重要な役割を持つp53アイソフォームの翻訳を強化する可能性が示唆された。

以上の研究は、細胞がん化に関わる p53 の制御機構の解明に貢献し、基礎生物学の発展に貢献すると評価できる。

したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和　5年　7月　20日実施の論文内容とそれに関連した試問を受け、提出されたレポートの内容の評価も併せて合格と認められたものである。