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〈一般論文〉

複製老化したヒト表皮角化細胞における ROS 発生の増大には ATP 産生能が低下したミトコンドリアの増加と Fe^{2+} の蓄積が関与する

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Involvement of Increased Numbers of Dysfunctional Mitochondria and the Accumulation of Fe^{2+} in the Enhanced Generation of ROS in Replicative Senescent Human Epidermal Keratinocytes

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Abstract

It is well known that the generation of reactive oxygen species (ROS) is increased in aged cells, however, there have been no reports on ROS levels in replicative senescent normal human epidermal keratinocytes (NHEKs). In this study, we investigated the mechanism of increased ROS generation in replicative senescent NHEKs using long-term cultured NHEKs. We defined primary NHEKs cultured for 27 days as aged NHEKs since they express various senescence markers and have decreased proliferation, and compared them with young NHEKs cultured for 13 days that did not show any senescence traits. The number of mitochondria in aged NHEKs increased along with cell enlargement due to aging but the mitochondrial density per protein content was not significantly different from that of young NHEKs. However, the ability to produce ATP was significantly decreased in aged NHEKs compared to young NHEKs. On the other hand, the amount of superoxide that was produced by mitochondria was not significantly different between young and aged NHEKs, and the amount of Mn-SOD (superoxide dismutase), which catalyzes superoxide to hydrogen peroxide, was significantly increased in aged NHEKs compared to young NHEKs. In addition, when hydrogen peroxide was added to the culture medium of young and of aged NHEKs, the scavenging rate was unexpectedly higher in aged NHEKs than in young NHEKs. Furthermore, the amounts of hydroxyl radicals derived from hydrogen peroxide, and Fe^{2+}, which catalyzes the reaction, were both higher in aged NHEKs than in young NHEKs. These results suggested that some hydrogen peroxide that had not been eliminated by catalase or glutathione peroxidase is converted to hydroxyl radicals by Fe^{2+}, which may contribute to the increased generation of ROS in replicative senescent NHEKs.

Key words:　ROS, replicative senescence, keratinocyte, mitochondria, hydrogen peroxide.