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学位論文名	Telomere dysfunction and inactivation of the p16^{INK4a}/Rb pathway in pyothorax-associated lymphoma （膿胸関連リンパ腫（PAL）におけるテロメア機能不全とp16^{INK4a}/Rb経路不活性化の関与）
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論文内容の要旨

The purpose of this study is to determine whether telomere dysfunction and inactivation of p16^{INK4a}/Rb pathway play a role for PAL development.

Pyothorax -associated lymphoma (PAL) is a non-Hodgkin lymphoma, mostly with B-immunophenotype . PAL is unique in that it developes in patients with more than 20 years history of chronic pyothorax resulting from artificial pneumothorax for the treatment of lung tuberculosis. PAL is strongly associated with Epstein Barr Virus (EBV) infection.

In 1987, PAL was initially described as a distinctive clinicopathologic entity by Dr. Aozasa Katsuyuki and later listed as disease entity in the WHO classification.

Telomeres are nucleoprotein complexes which locate at the end of eukaryotic chromosome. They consist of tandemly repeated DNA sequence (TTAGGG).

Human telomeres have various lengths ranging from 5 to 15 Kb.

The function of telomeres are (1) to prevent the terminal fusion of chromosome, (2) to protect the chromosome ends from destruction and (3) to control chromosomal position in the nucleus.

CDKN 2A gene locates on chromosome 9 p21 and encodes two distinct tumour suppressor genes p14 Arf and p16 ^{INK4a}. p14 Arf and p16^{INK4a} are involved in p53 and pRb tumour suppressive pathways. p16 ^{INK4a} inhibits phosphorylation of the pRb, thus prevents cell cycle progression. As a gatekeeper, p16 ^{INK4a} play a role for prevention of telomere-driven cytogenetic evolution.

Previous study indicates that telomere dysfunction induce chromosomal instability which occurs in the early phase of tumorigenesis. Chromosomal instability is induced before or soon after initial mutation or inactivation of tumour suppressor gene such as p16$^{INK\ 4a}$/Rb. Chromosomal instability then drives the multiple genetic changes that are required for the formation of tumours. Telomerase activation occurs in the late stage of tumorigenesis thus increases the replicative potential of tumor cells

Methods employed to determine the correlation between telomere dysfunction and p16 pathway inactivation in pyothorax -associated lymphoma, are (1) southern blotting and FISH hybridization to measure telomere length,(2)western blot and immunohistochemistry to show expression of p16^{INK4a}, p53, p14, pRb phosforylated and hTert protein, (3) Quantitative Telomerase Detection kit real time PCR assay to measure telomerase activity, and (4) bisulfite sequencing to examine methylation of p16 promoter region.

Result

Southern blotting demonstrated that telomere length in PAL cell lines as well as Hodgkin cell lines was shorter than that in Burkitt lymphoma. TL in clinical samples from PAL were various but extensively shortened than those in the peripheral blood leukocytes from the same patients.

Telomere shortening might make detection of the signal from telomere with FISH is difficult. This phenomenon is called telomere erosion. Loss of telomere signals was frequently found in the OPL-2, OPL-5, OPL-7, suggesting that the telomere length is shortened in PAL cell lines.

The mean telomerase activity in eight PAL cell lines was various, but not significantly different

from that in Burkitt lymphoma cell lines, Raji, Ramos and Namalwa..

Rb expression was detected in OPL-4,deglis and PAL-1 cell lines and 4 of 15 clinical samples from PAL, respectively. Protein $p16^{INK4a}$ expression was detected in neither any cell lines nor clinical samples. Rb protein expressed in 2 PAL cell lines was condensely phosphorylated,

Discussion

The possible molecular event undertaken in development of PAL

1. The causative factors for occurrence of telomere shorthening in PAL are (1) reactive oxygen species as a result of chronic inflammation (2) EBV Infection (3) recurrent chest rontgenogram for artificial pneumothorax.
2. Telomere shortening in B cell lymphocytes induce replicative senescence which is regulated by cell cycle check point protein particularly $P16^{INK\ 4a}$/Rb pathway.
3. Telomere shorthening cause telomere dysfunction, thus inducing genome instability.
4. Inhibition of $P16^{\ INK\ 4a}$/Rb pathway in PAL cell lines as well as in tumour samples allows continuous cell division and promotes telomere shortening.
5. Under crisis such condition, telomeres lose capping function, thus allow a noxious influence on the normal genome replication process resulting in genome instability.
6. Genome instability induce the reactivation of telomerase activity which is really important to restore genomic stability to a level permissive for cell viability and tumor progression.
7. It is matching with previous postulate that such dysfunctional telomere play a role in neoplastic transformation through induction of genome instability, therefore playing a causal role for PAL development
8. The reactivation of telomerase in PAL cell lines is important in the stabilization of telomere ends and allows continuous tumor growth.

論文審査の結果の要旨

膿胸関連リンパ腫(PAL) におけるテロメア機能不全と$p16^{INK4a}$/Rb 経路不活性化の関与についての研究である。

Pyothorax –associated lymphoma（以下、PALと呼ぶ）はnon― Hodgkinリンパ腫の一種で、２０年以上の経過をもつ慢性膿胸に続いておこるリンパ腫である。PAL は主として B免疫表現型 を示し、EBV 感染と強い相関 を有している。

これまで、PALでは様々な遺伝子に異常があることを、我々はPALより樹立した多くの細胞株を用いて報告してきた。本研究は、PALにおいて腫瘍発生に重要な役割を果たすテロメアが機能障害をおこしているか、さらにテロメアの機能障害が$p16^{INK4a}$/pRbの不活性化を引き起こすかを明らかにすることを目的とした。研究結果は PAL 細胞株と臨床材料で ﾃﾛﾒｱ長の短縮が、FISH 法で三つのPAL 細胞株（OPL2,5,7）でﾃﾛﾒｱsignalの消失がみられた。PAL細胞株は様々なﾃﾛﾒﾗｰｾﾞ活性を示したが、テロメア短縮との関係はなかった。PAL 細胞株と臨床材料において$p16^{INK4a}$/Rb 経路不活性化が観察されたが、三つのPAL 細胞株と四つの臨床材料では Rbの過剰リン酸化がみられた。過剰メチル化状態も全てのPAL 細胞株と四つの臨床材料でみられた。

$p16^{INK4a}$/Rb 経路不活性化は 細胞分裂を促進し、テロメアｸﾗｲｼｽをもたらすことから、ｹﾞﾉﾑ不安定性を誘導しその結果、ﾘﾝﾊﾟ腫の発生に至るものと推測される。膿胸関連リンパ腫(PAL)の発生においてテロメア機能不全と$p16^{INK4a}$/Rb 経路不活性化の役割が考えられる。

本研究は慢性炎症を基盤に発生するPALの発生機序の理解を進めるものであり。

学位授与の価値あるものと認める。