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博士の専攻分野の名称	博　士（医　学）
学 位 記 番 号	第　２１００２　号
学位授与年月日	平成 19 年 3 月 23 日
学位授与の要件	学位規則第４条第１項該当 医学系研究科未来医療開発専攻
学 位 論 文 名	Toll-like receptor 3 mediated hyperphosphorylation of tau in human SH-SY5Y neuroblastoma cells （ヒト SH-SY5Y 神経芽細胞種における Toll-like receptor 3 を介するタウ蛋白のリン酸化）
論文審査委員	（主査） 教　授　武田　雅俊 （副査） 教　授　祖父江憲治　　教　授　井上　洋一

論 文 内 容 の 要 旨

Neurofibrillary tangles (NFT) are neuropathological hallmarks of Alzheimer's disease (AD) and abnormally hyperphosphorylated tau is the major protein component of NFTs. In addition activation of immune cells like activated microglia and accumulation of inflammation associated proteins including various cytokines, complements are also a common phenomena associated with AD. The etiopathogenesis of hyperphosphorylation of tau has been studied for ages by number of investigators and numerous protein kinases and protein phosphatase have been implicated in the deregulation of tau phosphorylation in AD brain. A recent study has suggested hyperphosphorylation of tau in cortical neurons to be mediated by Interleukin-1 of activated microglia through p38-MAPK pathway thus linking activation of innate immune cell of CNS and hyperphosphorylation of Tau. The evolutionarily ancient innate immune system provides the first line of host defense against a large variety of pathogens, tissue insults and also controls many aspects of the adaptive immune response. Cells of the innate immune system recognize invariant pathogens associated molecular patterns through a series of genetically conserved and stable cell surface receptors related to the Drosophila gene toll that thus are referred to as Toll- like receptors (TLR). Broad expression of various TLR s (10 in number) has been already reported in human brain. Moreover activation of innate immunity in CNS is found to trigger neurodegeneration through a Toll like receptor 4 dependant pathway.

We focused on Toll like receptor-3 (TLR3) which has been shown to respond to its two known ligand, double stranded RNA (dsRNA) ; replication intermediary for many viruses and endogenous mRNA ; released from or associated with necrotic cells. Upon binding with its ligand, TLR3 has been shown to activate a variety of signaling pathways including activation of p38 MAP kinase and Jun N-terminal kinase (JNK). Moreover Subsclerosing Pan Encephalitis (SSPE), one of the known taupathies caused by measles Virus (RNA virus) has shown the evidence of neuronal loss, infiltration of inflammatory cells along with neurofibrillary tangles. Taken together, we hypothesized whether ligand mediated activation of TLR3 can induce hyperphosphorylation

of tau through activation of MAP kinases thus elucidating a mechanistic link between activation of innate immune receptor and NFT in the pathogenesis of various taupathies. In the present study we evaluated the expression level of TLR3 in Human SH-SY5Y neuroblastoma cell line and determined ligand induced activation of TLR3 to mediate hyperphosphorylation of tau.

Total RNA was isolated from SH-SY5Y cells and reverse transcriptase PCR was done to see endogenous expression of TLR-3 in SH-SY5Y cells that was further confirmed at protein level by western blot analysis. Cells were treated with 50 ug/ml of polyinosinic-polycytidylic acid (pIpC), a synthetic analogue of dsRNA and changes of phosphorylation of tau protein were investigated. Further the level of phosphorylation of tau protein was investigated when cells had been previously treated with 10 ng/ml of lipopolysaccharide (LPS) for 6 hours to induce over-expression of TLR-3. Increased phosphorylation of tau protein at PHF-1 site (Ser396/404) and activated JNK and p38 MAPK were observed in cells treated with pIpC. And these effects were enhanced when cells were pre-treated with LPS, a known transducer of TLR-3.

This study showed the evidence that TLR3 might be a potential mechanistic link between innate immunity and hyperphosphorylation of tau. A better understanding of how innate immunity affects hyperpshphorylation of tau and neurodegeneration will help to develop new diagnostic and therapeutic approach.

論文審査の結果の要旨

アルツハイマー病には、神経原線維変化、老人斑、緩徐進行性炎症性変化といった神経病理学的特徴があり、その中の神経原線維変化の主要構成成分としてリン酸化されたタウ蛋白がある。本研究では、近年見出された自然免疫に関わる分子である Toll-Like Receptor（TLR）に注目し、TLR3 とタウ蛋白リン酸化の関係について解析が行われた。そして、培養神経系細胞に Toll-Like Receptor3（TLR3）が発現していること、TLR3 に対する人工的リガンドである poly IC を添加すると p38 MAPK および JNK を介してタウ蛋白がリン酸化亢進すること、さらにアルツハイマー病脳において TLR3 の発現がきわめて亢進していることを見出した。このことより、アルツハイマー病の病態に TLR3 を介した細胞内情報伝達系が関与している可能性が示唆された。このことは、アルツハイマー病で認められる緩徐進行性炎症性変化とタウ蛋白リン酸化を病態的に結びつける重要な基本的知見を与え、病態の理解にきわめて重要な貢献を与えたものと考える。

よって、著者は博士（医学）の学位授与に値する。