NOTE

Expression, phosphorylation, localization, and microtubule binding of tau in colorectal cell lines

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Abstract Tau is a microtubule-associated protein that causes proper role of neuron by assembling and stabilizing microtubules. The amount and post translational modification of tau can change its function as a stabilizer of microtubules. The aim of the study was to look for the expression of tau in colorectal cancer and normal cells, along with phosphorylation and microtubule binding properties of tau expressed in colorectal cancer cell. Two colorectal cancer cells (SW480 and HCT 116) expressed tau that was also phosphorylated, whereas the others (Caco-2, HT-29 and DLD1) did not. Colorectal normal cell (CCD-18Co) expressed very tiny amount of tau that was not phosphorylated. A big fraction of tau in HCT 116 did not bind to microtubule. The results suggest that some colorectal cancer cells express hyperphosphorylated tau as found in Alzheimer's disease. So, tau in colorectal cancer cells does not look like same as tau in normal adult brain; rather it works nearly same as tau in neurodegenerative disease.

Keywords Cancer · Colorectal cells · Expression · Phosphorylation · Tau

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Introduction

Tau, that is copious in the central nervous system (CNS) and scarce elsewhere, is a heat stable microtubule-associated protein (MAP) and was discovered almost 40 years ago. In CNS, tau has 6 isoforms and controls microtubule assembly and polymerization along with regulate axonal diameter, axonal transport, neuronal polarity, and neurogenesis (Weingarten et al. 1975; Witman et al. 1976; Caceres and Kosik 1990; Harada et al. 1994; Dixit et al. 2008). The leading role of tau is its ability to assist microtubule assembly and stability (Weingarten et al. 1975; Lee et al. 2001). Besides neuron, tau expression has been proclaimed in different non-neuronal cells like liver, kidney, and muscle (Kenner et al. 1994; Gu et al. 1996). Tau has been also expressed in human breast, prostate, gastric, and pancreatic cancer (Sangrajrang et al. 1998; Rouzier et al. 2005; Mimori et al. 2006; Jimeno et al. 2007; Souter and Lee 2009). Tau from non-neuronal cells has similar structure and function with brain tau (Cross et al. 2000).

The microtubule assembly and stability of tau are conducted by the phosphorylation state of tau that is also regulated by kinases and phosphatases (Mandelkow et al. 1995; Liu et al. 2005). For immaculate function of tau, a normal phosphorylation level of tau is demanded; whereas if this typical phosphorylation level of tau is changed to hyperphosphorylated state, then the hyperphosphorylated tau fails to keep its biological activity (Kolarova et al. 2012). In age-related neurological disorders like Alzheimer's disease (AD), tau becomes aberrantly hyperphosphorylated (Kosik et al. 1986; Wood et al. 1986). Some cell cycle markers may be changed in some diseases linked with existing abnormal tau, indicating the presence of

anomalous tau responsible for the imperfect cell cycle re-entry (Andorfer et al. 2005).

Colorectal cancer is one of the dominant reasons of death by cancer among the people of western world (Jemal et al. 2003). As far as we know, however, there is no report on the expression of tau in colorectal cancer till now. Here, we investigated the expression, phosphorylation, and microtubule binding of tau in different colorectal cancer cell lines along with normal colorectal cell line. Our results demonstrated that tau is not only expressed but also phosphorylated in some of the colorectal cancer cell lines and pointed out that tau in colorectal cancer cells may have similarities with age-related dementia like AD in some aspects.

Materials and methods

Cell lines and cell culture

Human colorectal cancer cell lines (HCT 116, HT-29, Caco-2, DLD-1, and SW480) and human colorectal normal cell line (CCD-18Co) were purchased from ATCC (Manassa, VA, USA). Human neuroblastoma cell line (SH-SY5Y) was purchased from Korean Cell Line Bank (KCLB, Seoul, Korea). HCT 116, CCD-18Co, and SH-SY5Y cells were cultured in MEM (EBSS) medium (Hyclone, Logan, Utah, USA) with 10 % (v/v) fetal bovine serum (FBS) (Gibco, Carlsbad, CA, USA) and penicillin–streptomycin (Hyclone). Caco-2 and HT-29 cells were cultured in DMEM medium (Hyclone) with 10 % (v/v) FBS, non-essential amino acid (WelGene, Daegu, Korea), and penicillin–streptomycin (Hyclone). Also, DLD-1 and SW480 cells were cultured in RPMI medium (Hyclone) with 10 % (v/v) FBS and penicillin–streptomycin (Hyclone).

Antibodies

The mouse monoclonal antibody (mAb) of tau (tau-13) was bought from Abcam Inc. (Cambridge, MA, USA). The horseradish peroxidase (HRP)-linked anti-mouse IgG was purchased from Cell Signaling (Danvers, MA, USA). Also rabbit mAb of alpha-tubulin (11H10) and the HRP-linked goat anti-rabbit IgG were also bought from Cell Signaling. Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibody was purchased from Invitrogen (Carlsbad, CA, USA).

Western blot analysis for tau expression

For western blot analysis, the cultured cells were washed with PBS, harvested, and resuspended in cell lysis buffer plus PMSF, protease inhibitor, and phosphatase inhibitor

(Cell signaling) at 4 °C. Cell homogenates were centrifuged at $22,570 \times g$ for 25 min. Supernatant was taken, separated on 10 % SDS–polyacrylamide gel, and transferred to Immun-Blot PVDF membrane (Biorad, Hercules, CA, USA). The membranes were incubated with mouse anti-tau antibody (1:5000; Abcam) and rabbit anti-α-tubulin antibody followed by incubation of relevant secondary antibody conjugated with HRP. The signals on membrane were developed by SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, Waltham, MA, USA) and pictured by LAS-4000 Luminescent image analyzer (Fujifilm Co. Ltd, Tokyo, Japan).

Phosphatase treatment

From the cell lysates that were prepared for tau expression, the proteins were treated with thermosensitive alkaline phosphatase (Thermo Scientific FastAP Alkaline Phosphatase; Thermo Fisher Scientific) that contains alkaline phosphatase and alkaline phosphatase buffer. Cell lysates that contain enough protein were added with alkaline phosphatase and alkaline phosphatase buffer, and also water if need. Tapping a couple of times to mix properly and incubation at 37 °C for 1 h were done. Then sample buffer was introduced and heated at 100 °C for 5 min to stop the reaction. For control of each sample, samples having same amount of protein were prepared. As a reference of dephosphorylated tau, tau ladder (Sigma-Aldrich, St Louis, MO, USA), which was prepared in *Escherichia coli* and had 6 isoforms, was used.

Immunofluorescence

HCT 116, HT-29, SW480, Caco-2, CCD-18Co, and SH-SY5Y cells were grown on cover glass placed in 6-well plates. The cells were fixed with 3.7 % paraformaldehyde, permeabilized with 3 % BSA and 0.1 % Triton X-100 in PBS for 10 min, and blocked with 3 % BSA in PBS solution for 1 h at room temperature. The cells were probed with mouse anti-tau (tau-13) mAb (Abcam-ab24634) in PBS with 1 % BSA and 0.1 % Triton X-100 (1:500 dilution) and visualized with Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibody (1:500 dilution). Cells were washed with PBS again and mounted with DAPI (VECTASHIELD, Vector laboratories, Burlingame, CA, USA) for 1 h at dark humid chamber. Leica TCs SP5 confocal microscope (Leica Microsystems AG, Wetzlar, Germany) was used to take the image.

Microtubule (MT) binding

Taxol-stabilized and lyophilized MTs and MAP fraction of bovine brain were purchased from Cytoskeleton (Denver, CO, USA). MT binding assay of colorectal cancer cells that

Fig. 1 Expression (**A**) and phosphorylation (**B**) of tau in different colorectal cell lines. (**A**) Expression of tau in different colorectal cell lines. (**B**) Odd lanes showed control incubation, whereas even lanes showed phosphatase treatment. Phosphatase treatment showed increased mobility of tau. Six isoforms of tau synthesized in *E. coli* were run as comparison (tau ladder)
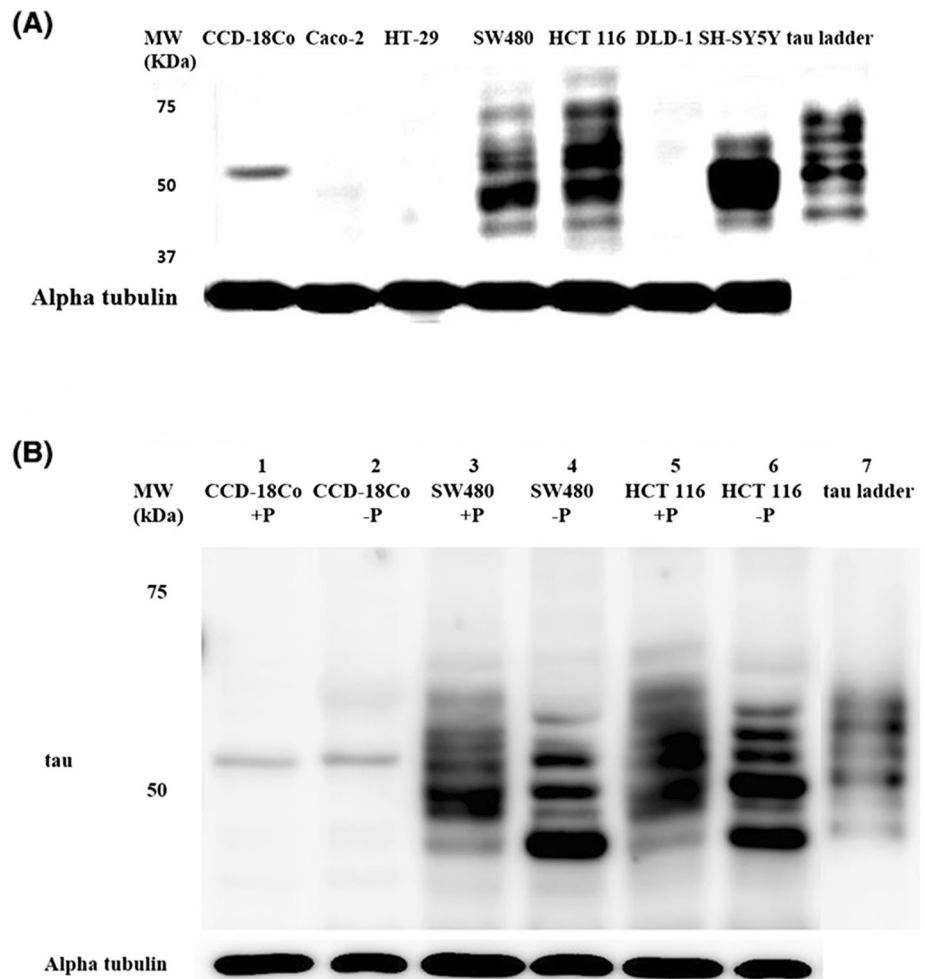
express tau, along with neuroblastoma cell, was done as previously described by Souter (Souter and Lee 2009). The microtubules were pelleted. Supernatant containing unbound tau was removed, and equal volume of sample buffer was added to pellet as supernatant and check the protein concentration. Both fractions of pellet and supernatant were immunoblotted and compared.

## Results

Expression of tau

Though tau is a neuronal protein, it is also found in different cancer cell lines of organs like breast (Rouzier et al. 2005), prostate (Sangrajrang et al. 1998; Souter and Lee 2009), stomach (Mimori et al. 2006), and pancreas (Jimeno et al. 2007). Here we tried to search the expression of tau in different colorectal cancer cell lines (HCT 116, HT-29, SW480, Caco-2, and DLD-1) along with non-cancerous colorectal cell line (CCD-18Co) as well. Vast level of tau

was detected on SW480 and HCT 116, and very truncated level of tau was detected on CCD-18Co (Fig. 1A). Caco-2, HT-29, and DLD-1 did not express tau even when the blot was intentionally overexposed (data not shown). As the expression of fetal tau isoform was reported in human neuroblastoma cell (SH-SY5Y) (Smith et al. 1995), we compared the level of tau expression of SH-SY5Y with colorectal cell lines. We showed that colorectal cell lines exhibited a broader assortment of tau and different expression levels of total tau.

Phosphorylation of tau

To check the possible phosphorylation of colorectal tau, we treated tau with alkaline phosphatase. As we know that phosphorylated protein moves slower than non-phosphorylated one, we can pattern phosphorylation of protein by phosphatase treatment. By phosphatase treatment, tau of colorectal cancer cells migrated faster (Fig. 1B, Lanes 3–6) whereas that of colorectal non-cancer cell did not (Fig. 1B, Lanes 1–2).

Fig. 2 Localization of tau in colorectal cancer and normal cell lines. Confocal immunofluorescence of anti-tau antibody in HCT 116, SW480, HT-29, Caco-2, CCD-18Co, and SH-SY5Y cells was shown in green. Cell nuclear was stained with *blue*
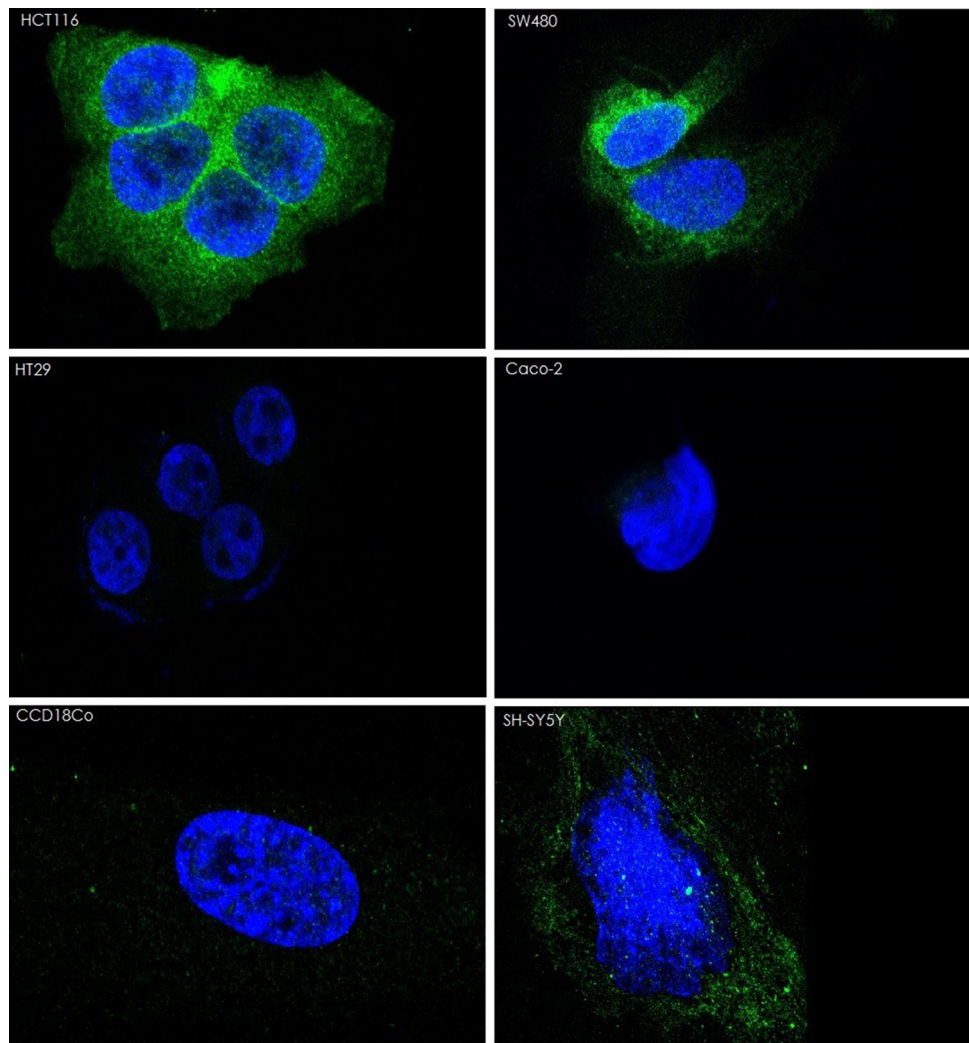
Localization of tau

Using confocal microscopy, the localization and expression of tau were observed in colorectal cancer cells along with the normal cell. Tau with microtubules appeared at cell boundary. Unbound tau was clearly apparent in HCT 116 and SW480 (Fig. 2). Low amount of tau was observed in CCD-18Co as shown in Fig. 2 as well as in Fig. 1. In HT-29 and Caco-2, tau expression was not observed. Moderate level of tau was detected in neuroblastoma cell SH-SY5Y (positive control).

Microtubule binding of tau

To compare the microtubule binding of HCT 116 tau with other tau, tau from HCT 116, SH-SY5Y, and *E. coli*, and bovine brain MAPs were subjected to microtubule binding assay. Tau from *E. coli* and MAPs from bovine brain were mainly bound to microtubules. In HCT 116 and SH-SY5Y, a large proportion of tau existed as unbound (Fig. 3).

Discussion

Till now, there is no report published on tau in colorectal cancer. Tau levels might be related to the resistance of various cancers to anti-mitotic drugs (Rouzier et al. 2005; Mimori et al. 2006; Jimeno et al. 2007). Our hypothesis was that tau might be expressed and phosphorylated in some colorectal cancer cells like other cancers and AD.

In case of breast cancer, 52 % of patients are tau negative (Rouzier et al. 2005); nearly same result (57 %) was demonstrated by a different research group (Pusztai et al. 2009). 30 % of patients are tau negative in gastric cancer (Mimori et al. 2006) and 25.7 % of patients are tau negative in ovarian cancer (Smoter et al. 2013). These results indicate that tau expression may be different depending on the type of cancer. However, additional research is needed to figure out tau expression according to the different stage in the same type of cancer. In our experiment, we have compared the expression of tau in different colorectal cancer cell lines (genetic phenotype, ploidy, and Duke

Fig. 3 MT binding of tau in HCT116 cell line protein from HCT 116 and SH-SH5Y cells, *E. coli* tau and bovine brain MAPs were incubated with microtubules as illustrated in materials and methods. The same proportions of supernatant (S) and pellet (P) fractions of tau were immunoblotted with anti-tau mAb. Control lanes of MT only (lanes 9–10) were run to confirm that MT does not have any endogenous tau
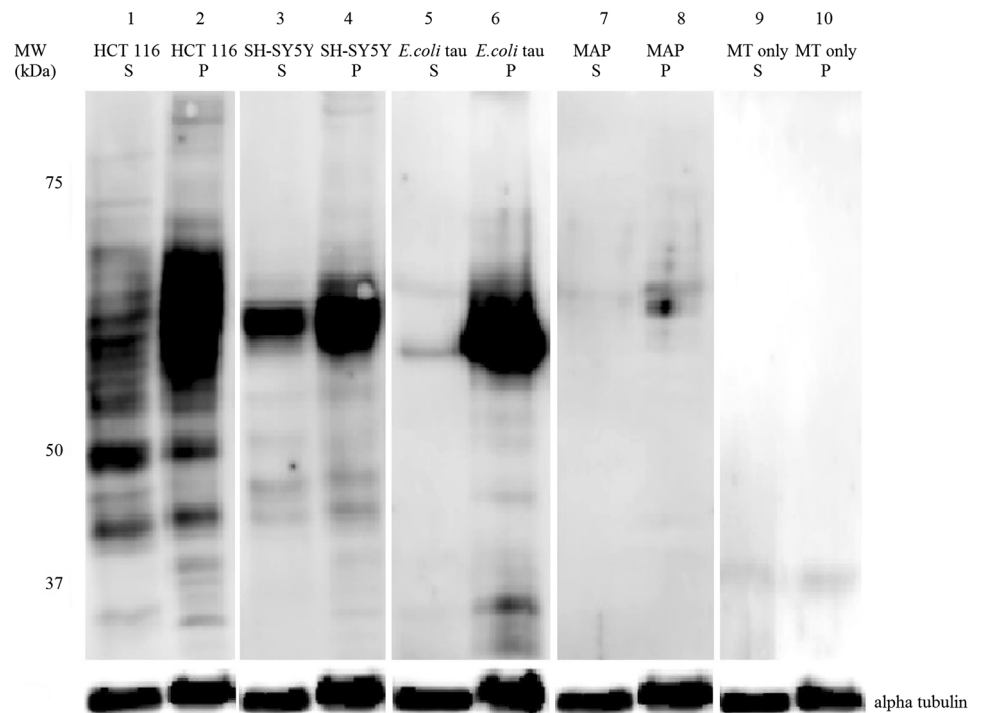
stage) along with colorectal normal cell line mainly at protein level. Out of five colorectal cancer cell lines, SW480 and HCT 116 expressed high levels of tau, which were confirmed by immunofluorescence experiment. The mechanism by which tau expression is regulated differently in each colorectal cancer requires further investigation. As there are a lot of neuronal and non-neuronal cell lines not expressing different tau isoforms (Smith et al. 1995), the expression of different tau isoforms in HCT 116 marks an auspicious cell to explore human colorectal tau. SW480 and HCT 116 showed different patterns of tau expression and phosphorylation, whereas CCD-18Co expressed only tiny amount of tau that was not phosphorylated.

The activity of tau is controlled by its phosphorylation. The phosphorylation of tau normally occurred in neurodegenerative disease. Our experimental data showed that human colorectal cancer tau was also phosphorylated like neurodegenerative disease such as AD. However, colorectal normal cell that expressed a very low level of tau was not phosphorylated as like normal adult brain tau. In this aspect, we can get some similarities of neurodegenerative disease such as AD with colorectal cancer.

Hyperphosphorylation lowers the activity of tau to endorse microtubule assembly C in AD (Lindwall and Cole 1984; Alonso et al. 1994, 1996). In this study, tau expressed in colorectal cancer cell lines was hyperphosphorylated, and a bulky proportion of the tau did not bind to microtubules. In some studies of tau in different cancers, there had been a relationship with tau expression and drug

resistance (Rouzier et al. 2005; Wagner et al. 2005; Souter and Lee 2009; Li et al. 2013; Smoter et al. 2013; Wang et al. 2013). When phosphatase and tensin homolog, a tumor suppressor, lost phosphatase activity, tau was hyperphosphorylated resulting in tau aggregation (Zhang et al. 2006). The exact mechanism of how hyperphosphorylation of tau affects the occurrence and progression of colorectal cancer is a topic of further research. The regulation of expression and phosphorylation of tau in SW480 and HCT 116 might be applied to the therapeutic strategy of colorectal cancer through the further study.

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