Role of CMT2, mitotic checkpoint regulator, in tumor cell

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1. Introduction

A principal therapeutic intent of exposing a tumor to ionizing radiation is to produce irreversible DNA damage in the tumor cells while sparing the around normal tissues involved. The fast kinetics of cell cycling in the tumor cells decreases the desired-effect and requires reduction in overall treatment time to achieve destruction of the malignant cells with minimal adverse effects on the surrounding non-malignant tissue. The access of molecular cell cycle-targeted therapy may represent a supplement to accelerated fractionation regimens in improving the therapeutic index.

Complete depletion of Mad2 protein levels induces mitotic failure, multi-nucleation, and apoptosis in tumor cell utilizing RNA interference [1]. CMT2 counteracts the function of Mad2 and is required for the silencing of the spindle checkpoint [2].

In this study, we identified that CMT2 induces tumor cell death and permanent growth arrest.

2. Methods and Results

Cells were seeded into 24-well plates in RPMI culture medium including 10% FBS. After 24 h, cells were infected with retrovirus including CMT2 gene, and the cells were incubated for 1~9 days. Cells were fixed for 5 min in 3% formaldehyde, washed, and incubated at 37°C with SA-β-gal staining solution (pH 6.5). After 24 h incubation, cells were stained with DAPI for identification of nuclear morphology. Photographs were taken with a light and fluorescence microscope.

To analyze the possible functional effects of CMT2, we introduced the full-length human gene into a bicistronic retroviral expression system co-expressing EGFP or puromycin as a selectable marker. The resulting constructs, termed CMT2-IRES-EGFP and CMT2-IRES-puro, were retrovirally transduced into cancer cell lines. The infectivity of CMT2-IRES-EGFP and corresponding control-IRES-EGFP retroviral constructs was approximately >89%, as determined by counting cells displaying green fluorescence.

2.1 p31 reduce clonogenic ability of variety cancer cell lines

At first, we examined whether CMT2 influences clonogenic activity of variety cancer cell lines as well as proliferation. Transduction of CMT2 led to a significant reduction in formation of colony, while the control vector did not affect the growth rate (Table 1). Cells transduced with the CMT2–IRES-EGFP retroviral vector formed only some colonies, none of which were EGFP-positive, except HepG2 and H460, indicating that the cells transduced with CMT2 did not form colony. In contrast, control-IRES-EGFP retroviral vector transduction produced 80~90% EGFP-positive of total colonies. In view of this finding, we propose that CMT2 elicits complete regression of colony formation.

Table 1. Effect of human CMT2 on colony formation of a variety cell lines

	hp31comet			Control		
	Colony No. GFP(+)	Total	Infectivity (%)	Colony No. GFP(+)	Total	Infectivity (%)
Lung						
A549	0	0.3	93	226.3	252	95.5
Calu-1	0	34.7	72.1	189.7	197.7	80.2
Sk-Lu-1	0	2.7	89	172	187	96
H460	25	45.7	85.4	152	178.3	63.6
H446	0	0	93	154.7	160.3	95.3
Cervix						
HeLa	0	2.3	87.2	242.7	285	86.3
SW756	0	155.7	48.2	186.3	299	66.7
SiHa	0	2.7	67.2	194.7	283.7	67.2
MS751	0	37	52.3	108.3	172	53.4
Caski	0	2.7	84.3	159	165.2	84.9
Breast						
MCF-7	0	55.9	45.5	270.3	385.3	75.4
Liver						
Sk-Hep-1	0	148.7	57.9	115	195	53.3
Chang	0	42	73.7	177	248.3	85.6
Hep3B	0	143.7	48.4	61	235.7	35.7
Huh-7	0	17.7	67.9	61.3	96.7	73.3
HepG2	44.7	70	89.3	180	196.7	95.3
Bone						
SaOs-2	0	126.7	51.2	120.7	268	56.8
U-2OS	0	2.3	91.7	188.7	200.3	86.2
Kidney						
293	0	29.7	82.4	193.3	243	80.6

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2.2 CMT2 suppresses tumor cells by multiple-way

As shown in Figure 1A, colony number of CMT2 overexpressed-A549 cell was markedly decreased as well as other cancer cell lines. In response to overexpression of CMT2 in A549, Calu-1 and U2OS, we observed profound alterations in both cell proliferation and morphology. While control cell lines grew as small, rounded, refractile cells, the overexpressed CMT2 caused cells to exhibit increased size and flattened morphology (Fig 1A). The frequencies of SA-b-gal positive cells reached up to 50% in the CMT2 induced three cell lines, as well as 82% abnormal nuclear (Fig 1B). However, large amounts of HeLa, Hep3B cells underwent cell death, including apoptosis and necrosis, upon CMT2 overexpression (Fig. 1C). In contrast to plenty of the molecular association between abnormal spindle checkpoints and apoptosis, there is a limited understanding of its molecular link directly to

senescence, as it has recently been reported in the cases of BubR1 insufficiency [3, 4] and Bub3/Rae1 double haploinsufficiency [5].

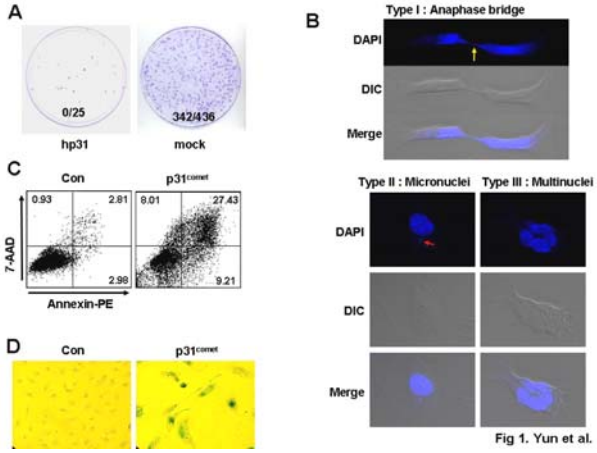

Fig. 2. Extotic expression of CMT2 inhibits clonogenic ability of cancer cell lines and induces apoptosis and/or senescence in a variety of cancer cell lines.

3. Conclusions

Induction of CMT2, silencing the spindle checkpoint by holding Mad2 function, drives cancer cell lines, A549, U2OS and Calu-1 to be irreversible growth arrest. Exogenously expressed CMT2 also induces cell death in other cancer cell lines, HeLa and Hep3B. And we could observe phenomena, senescence and apoptosis, for the most part of cancer cell lines at the same time. In transformed foci forming assay, MEFs were co-transfected with human *CMT2* or murine *CMT2*, *myc* and *Ras*, foci number of transformed cells is dramatically decreased than control vector transfected MEF. Above results distinctly mean that CMT2 can suppress proliferation of various established cancer cell lines. So, targeting of p31 may be exploited to increase the efficiency of radiation therapy.

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