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Short communication

Over-expression of RAD51 or RAD54 but not RAD51/4 enhances extra-chromosomal homologous recombination in the human sarcoma (HT-1080) cell line

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ABSTRACT

RAD51 and RAD54, members of the RAD52 epistasis group, play key roles in homologous recombination (HR). The efficiency of homologous recombination (HR) can be increased by over-expression of either of them. A vector that allows co-expression of RAD51 and RAD54 was constructed to investigate interactions between the two proteins during extra-chromosomal HR. The efficiency of extra-chromosomal HR evaluated by GFP extra-chromosomal HR was enhanced (110–245%) in different transfected Human sarcoma (HT-1080) cell colonies. We observed that RAD51 clearly promotes extra-chromosomal HR; however, the actions of RAD54 in extra-chromosomal HR were weak. Our data suggest that RAD51 may function as a universal factor during HR, whereas RAD54 mainly functions in other types of HR (gene targeting or intra-chromosomal HR), which involves interaction with chromosomal DNA.

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Homologous recombination (HR) is a phenomenon conserved during the cell cycle and is a major mechanism for maintaining the integrity and genetic diversity of heritable material (Khanna and Jackson, 2001). As a complex process, many proteins are involved in HR pathway (Sung and Klein, 2006), such as BRCA1-2, XRCC2-3, DMC1 and RAD52 epistasis group. The roles of the RAD52 epistasis group, including RAD50, RAD51, RAD52, RAD54, RAD55, and RAD57, were clarified during HR in eukaryotes. Especially the RAD51, RAD52 and RAD54 protein are key proteins in the HR process. Many studies had focused on RAD51 and RAD54 due to their important roles in HR process (Sung et al., 2003; Symington, 2002; Tan et al., 2003). RAD51 (RecA is a homologous protein in *Escherichia coli*) is a central protein in the events of HR (Bianco et al., 1998b). Biochemical analyses have shown that RAD51 is critical for forming the nucleoprotein filament (Ogawa et al., 1993), homology searching (Baumann et al., 1996), and leading strand exchange between recombining DNA molecules (Bianco et al., 1998a). RAD51 is essential for the cell survival in eukaryotes. RAD51 mutant is non-viable in mouse and chicken DT40 cell line (Sonoda et al., 1998; Tsuzuki et al., 1996). D.G. Schaefer et al. (2010) successfully obtained RAD51 mutant in moss *Physcomitrella Patens* and for the first time they demonstrated that the important function of RAD51 for HR in vivo. RAD54 (Rdh54 is a homologous protein in *Saccharomyces cerevisiae*) is an important recombination mediator in HR (Heyer et al., 2006) and works in concert with RAD51. RAD54 stabilizes the RAD51 filament independent of its ATPase activity (Wolner and Peterson, 2005), enhances the ability of the RAD51 filament to form joint molecules (Mazin et al., 2003), overcomes kinetic impediments (such as the nucleosome) for pairing of homologous DNA (Petukhova et al., 1998), changes the topological structure of the DNA duplex (Jaskelioff et al., 2003) and enhances the accessibility of chromosomal DNA. During the late stage of HR, RAD54 promotes branch migration (Solinger and Heyer, 2001) and may assist with releasing the RAD51 filament from duplex DNA at the end of HR (Solinger et al., 2002). RAD54 deficient mice are hypersensitive to the interstrand DNA crosslinking compound mitomycin C. However, the hypersensitivity to ionizing radiation is different during the development of mice (Essers et al., 2000).

Gene over-expression is a reasonable strategy for investigating functions of RAD52 epistasis in vivo. Many studies have focused on variation efficiency of HR or change of radiation hypersensitivity after over-expressing recombination mediators genes, including RAD51 (Arnaudeau et al., 1999; Kim et al., 2001; Vispe et al., 1998), RecA (RAD51 counterpart in yeast) (Scherbakova et al., 2000), RAD52 (Johnson et al., 1996; Park, 1995), and RAD54 (Shaked et al., 2005), their results showed that the rate of HR and/or resistance to ionizing radiation is enhanced in cell line or mice. However, few of studies have evaluated the cooperation among the recombination mediators.

Abbreviations: HR, homologous recombination; RAD51/4, RAD51 and RAD54.

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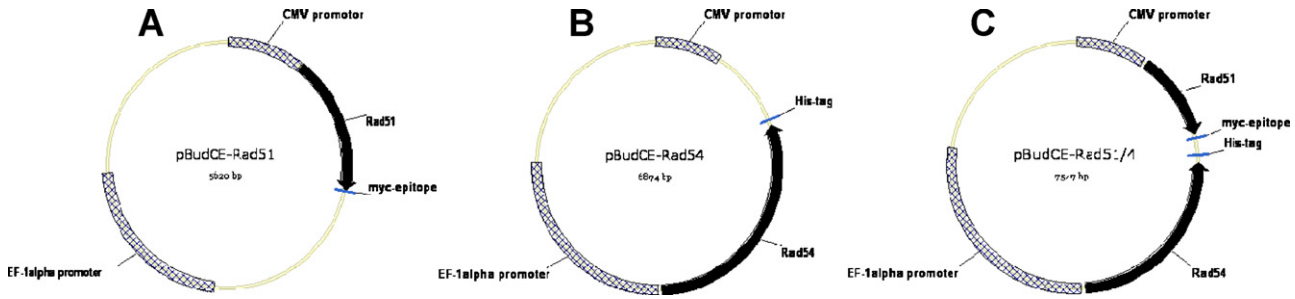

Fig. 1. Vectors for transgenes. (A) Human *RAD51* is driven by the CMV promoter, and is tagged by the myc-epitope at the C-terminal. (B) Human *RAD54L* is driven by the EF-1 alpha promoter, and is tagged by His-tag at the C-terminal. (C) The Rad51 and Rad54L co-expression vector, which combines the vectors in A and B. All vectors were derived from pBudCE4.1.

In the present report, we focused on whether cooperation between RAD51 and RAD54 proteins is an alternative way for enhancing the efficiency of HR in human sarcoma (HT-1080) cell. Three expression vectors-pBudCE-RAD51 (Fig. 1A), pBudCE-RAD54 (Fig. 1B), and pBudCE-RAD51/4 (co-overexpression of RAD51 and RAD54) (Fig. 1C) – were constructed (see Supplementary data). The GFP extra-chromosomal HR (Yanez and Porter, 2002b) (see Supplementary data) was adapted to assess the effect of human RAD51 and RAD54 over-expression on extra-chromosomal HR (Fig. 2).

We obtained several stable transgenic cell colonies expressing RAD51, RAD54, or RAD51/4 (see Supplementary data). Three stable cell colonies expressing each transgene were chosen to examine protein expression. As the coding regions of RAD51 and RAD54 were linked to a myc-tag and a His-tag, respectively, the products of the RAD51 and RAD54 transgenes were larger than their endogenous counterparts (Fig. 3).

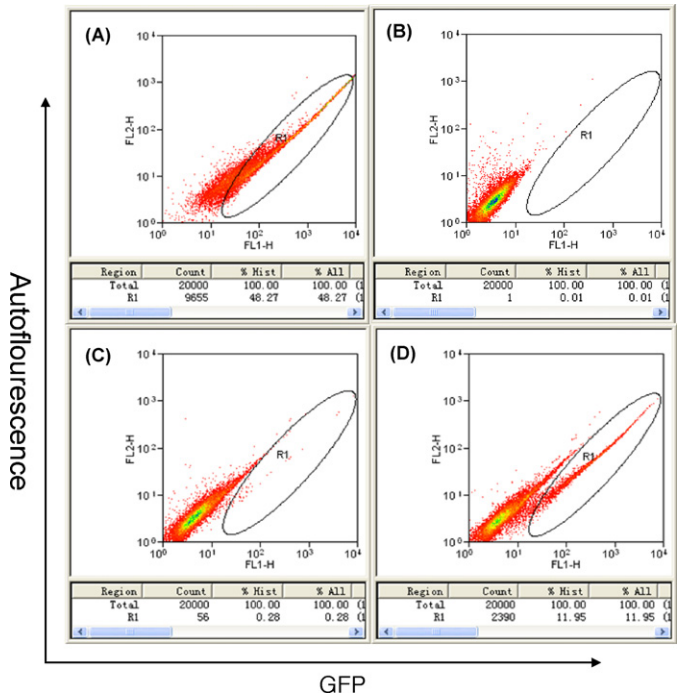

Fig. 2. FACS analysis. (A) The positive control: Cells were transfected with sGFP vector. (B) The negative control: Cells were transfected with sGFP-I vector. (C) The negative control: cells were transfected with linearized sGFP-PL vector. The sGFP-PL vector was linearized by XhoI before transfection to reduce the background signal. (digested with XhoI). (D) In the experiment, cells were co-transfected with linearized sGFP-PL and sGFP-I vectors. The total events were 2 × 104.GFP-positive cells were bracketed in region R1.

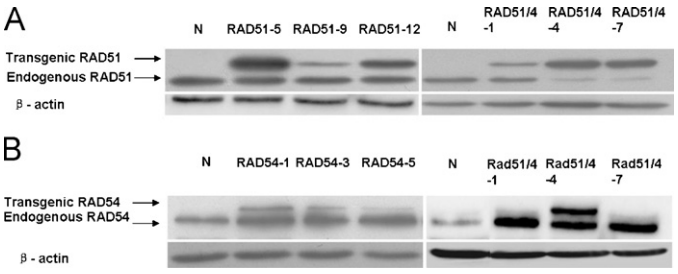

Fig. 3. Western blotting of the Rad51, Rad54, and Rad51/4 transgenes. (A) Western blotting analysis of the Rad51 expression in different cell colonies. Rad51-5, -9, and -12 are the Rad51 transgenic colonies, and Rad51/4-1, -4, and -7 are the Rad51 and Rad54 co-expressing colonies. (B) Western blotting analysis of the Rad54 expression in different cell colonies. Rad54-1, -3, and -5 are the Rad54transgenic colonies, and Rad51/4-1, -4, and -7 are the Rad51 and Rad54 co-expressing colonies.
N: pBudCE4.1 transfected clone. β-actin was used as a loading control.

FACS analysis was used to evaluate the effect of RAD51 and/or RAD54 over-expression on the efficiency of extra-chromosomal HR in different stable transgenic cell colonies (see Supplementary data). The relative efficiency of extra-chromosomal HR in the different cell colonies (nine transfected cell colonies (RAD51-(5, 9, 12), RAD54-(1, 3, 5) and RAD51/4-(1, 4, 7) and one negative control (pBudCE4.1 blank vector transfected cell colony)) is shown in Fig. 4. In the RAD51 transgenic colonies, the extra-chromosomal HR efficiency was significantly elevated and positively correlated with the expression level of recombinant RAD51 (Fig. 3, Fig. 4). Among the RAD54 transgenic clones, the extra-chromosomal HR efficiency was not enhanced significantly, except for in the RAD54-1 clone, in which a relatively high level of recombinant RAD54 was expressed. In the RAD51/4-4 colony, the RAD51 and RAD54 recombinant proteins were both expressed at high levels, but the efficiency of HR was not enhanced compared to that of the control. In contrast, the efficiency of HR was enhanced significantly

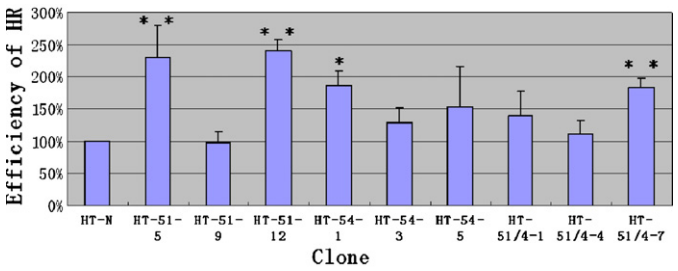

Fig. 4. Relative efficiency of extra-chromosomal HR in different clones. The efficiency of extra-chromosomal HR in different clones 24 h post-transfection was compared to the negative control. The error bars represent standard deviations ($n = 3$). * and ** indicate statistically significant differences compared to the negative control: $P < 0.05$ and $P < 0.01$, respectively.