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Suppression of breast cancer cell growth by Na^+/H^+ exchanger regulatory factor 1 (*NHERF1*)

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Abstract

Introduction Na^+/H^+ exchanger regulatory factor 1 *(NHERF1*, also known as *EBP50* or *NHERF*) is a putative tumour suppressor gene in human breast cancer. Located at *17q25.1*, *NHERF1* is frequently targeted during breast tumourigenesis. Loss of heterozygosity (LOH) at the *NHERF1* locus is found in more than 50% of breast tumours. In addition, *NHERF1* is mutated in a subset of primary breast tumours and breast cancer cell lines. LOH at the *NHERF1* locus is strongly associated with aggressive features of breast tumours, implicating *NHERF1* as a haploinsufficiency tumour suppressor gene. However, the putative NHERF1 tumour suppressor activity has not been functionally verified.

Methods To confirm the NHERF1 tumour suppressor activity suggested by our genetic analyses, we used retrovirus-transduced short hairpin RNA (shRNA) to knock down NHERF1 expression in breast cancer cell lines MCF7 and T47D. These cells were then assessed for cell growth *in vitro* and *in vivo*. The control and NHERF1 knockdown cells were also serum-starved and re-fed to compare their cell cycle progression as measured by fluorescence-activated cell sorting analyses.

Results We found that downregulation of the endogenous NHERF1 in T47D or MCF7 cells resulted in enhanced cell proliferation in both anchorage-dependent and -independent conditions compared with that of the vector control cells. NHERF1 knockdown T47D cells implanted at mammary fat pads of athymic mice formed larger tumours than did control cells. We found that serum-starved NHERF1 knockdown cells had a faster G_1-to-S transition after serum re-stimulation than the control cells. Immunoblotting showed that the accelerated cell cycle progression in NHERF1 knockdown cells was accompanied by increased expression of cyclin E and elevated Rb phosphorylation level.

Conclusion Our findings suggested that the normal NHERF1 function in mammary epithelial cells involves blockage of cell cycle progression. Our study affirmed the tumour suppressor activity of NHERF1 in breast which may be related to its regulatory effect on cell cycle. It warrants future investigation of this novel tumour suppressor pathway in human breast cancer which may turn up therapeutic opportunities.

Introduction

Na^+/H^+ exchanger regulatory factor 1 (*NHERF1*, also known as *EBP-50* or *NHERF*) is a candidate tumour suppressor gene in human breast cancer [1]. We reported loss of heterozygosity (LOH) at the *NHERF1* gene locus (*17q25.1*) in more than 50% of human breast tumours. Such loss is infrequent, however, in other tumour types, suggesting that *NHERF1* is specifically targeted during mammary tumourigenesis. In a panel of breast tumours pre-screened for LOH, three intragenic mutations of *NHERF1* were found (approximately 3%) [1]. LOH at the *NHERF1* locus is positively correlated with aggressive features of breast tumours, including tumour size, grade, and stage. The association indicates a critical role for *NHERF1* in mammary carcinogenesis, in which its putative suppressor activity is haploinsufficient. The haploinsufficiency of the *NHERF1* gene may explain its relatively low frequency of intragenic mutations.

ER = oestrogen receptor; ERE = oestrogen response element; ERM = ezrin-radixin-moesin; FACS = fluorescence-activated cell sorting; FBS = foetal bovine serum; LOH = loss of heterozygosity; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NF2 = neurofibromatosis-2; NHE3 = Na^+/H^+ exchanger isoform 3; NHERF1 = Na^+/H^+ exchanger regulatory factor 1; PBS = phosphate-buffered saline; PDGFR = platelet-derived growth factor receptor; PDZ = PSD-95/Dlg/ZO1; PTEN = phosphatase and tensin homologue (mutated in multiple advanced cancers 1); shRNA = short hairpin RNA; SYK = spleen tyrosine kinase.