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Establishment of an apoptosis-sensitive rat mammary carcinoma cell line with a mutation in the DNA-binding region of p53

Tetsuya Hamaguchi[a], Yoichiro Matsuoka[b,*], John Bechberger[c], Takamasa Ohnishi[d], Ken-ichi Fujita[e], Christian C. Naus[c], Masato Kusunoki[a], Airo Tsubura[b], Hiroyuki Tsuda[f,**]

[a]*Second Department of Surgery, Mie University School of Medicine, 2-174 Edobashi, Tsu, Mie 514-0001, Japan*
[b]*Second Department of Pathology, Kansai Medical University, 10-15 Fumizono-cho, Moriguchi, Osaka 570-8506, Japan*
[c]*Department of Anatomy, University of British Columbia, Vancouver, BC, Canada V6T 1Z3*
[d]*Department of Molecular and Environmental Pathology, The University of Tokushima Graduate School of Medical Sciences, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan*
[e]*Experimental Pathology and Chemotherapy Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan*
[f]*Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences, 1 Kawazumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan*

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Abstract

Seven mammary carcinoma cell lines were established from 7,12-dimethylbenz[a]anthracene-induced tumors developed in a human *c-Ha-ras* transgenic rat. Without apoptotic stimuli, a large amount of p53 protein was detected in the C11 cell line (C11), whereas all cell lines expressed variable levels of the assayed death receptor/ligand, *bcl-2* family and p53 cascade-related genes. The *p53* gene in C11 had a mutation at codon 246, in the DNA-binding region of p53. Transcriptional activity of the mutant protein appeared to be lower than that of the wild-type p53. Despite the presence of p53 mutation, C11 was more sensitive to apoptosis triggered by etoposide, paclitaxel and staurosporine than the cell lines expressing wild-type p53. These data suggest that the apoptosis induced by intracellular injury occurs via the transcriptionally impaired mutant p53 in C11.

Keywords: Apoptosis; Bcl-XL; Mammary carcinoma; p53; ras; Transgenic rat

1. Introduction

Apoptosis can be induced by two major pathways. The extrinsic pathway is triggered by extracellular death ligands such as the tumor necrosis factor (TNF) relatives Fas ligand and TNF-related apoptosis-inducing ligand (TRAIL), which bind Fas and DR4

* Corresponding authors. Tel.: +81 6 6993 9432; fax: +81 6 6992 5023.
** Tel.: +81 52 853 8991; fax: +81 52 853 8996
E-mail addresses: matsuoyo@takii.kmu.ac.jp (Y. Matsuoka), htsuda@med.nagoya-cu.ac.jp (H. Tsuda).

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or DR5, respectively [1]. The intrinsic pathway is initiated by intracellular injury such as DNA damage, which induces caspase activation via the Bcl-2 gene family [2,3]. Many physical and chemical DNA-damaging agents used in cancer therapy are potent activators of the tumor suppressor p53. Activation of p53 results in the expression of a number of genes that are involved in both major apoptotic pathways, including genes encoding pro-apoptotic members of the Bcl-2 family [4,5] and death receptors [6,7]. Thus, p53 plays roles in both the intrinsic and extrinsic apoptotic pathways.

The p53 tumor suppressor is mutated in over 50% of human cancers. The majority of these gene alterations are missense mutations, which often cause single-residue changes in the conserved DNA-binding core domain (residues 102–292 in human p53) of the protein [8]. Some common mutations in the DNA-binding domain lead to loss of the transcription- and apoptosis-inducing activity of p53. On the other hand, some transcriptionally inactive forms of p53 have been shown to mediate apoptosis via a transcription-independent mechanism [9–12]. Most recently, p53 has been shown to activate the pro-apoptotic protein Bax via binding to the anti-apoptotic proteins Bcl-XL and Bcl-2, resulting in mitochondrial membrane permeabilization and induction of apoptosis without target gene expression [13,14].

We have generated a rat strain that carries the human *c-Ha-ras* protooncogene and exhibits increased susceptibility to chemical carcinogens that target the mammary gland, urinary bladder and skin [15–17]. All of these transgenic (Tg) rats develop pre-neoplastic mammary lesions within 20 days after injection of *N*-methyl-*N*-nitrosourea (MNU) [18], and mammary carcinomas appear within 8 weeks after treatment with a variety of chemical carcinogens including MNU and 7,12-dimethylbenz[a]anthracene (DMBA) [15,19]. Interestingly, these carcinomas contain activating mutations preferentially in the human transgene [15]. These Tg rats also spontaneously develop alveolar hyperplasia and adenocarcinomas. Elevated expression of the *c-Ha-ras* protooncogene, rather than mutations in this gene, appears to be sufficient to cause a highly proliferative phenotype of mammary alveoli [20]. On the other hand, mammary tumorigenesis in these Tg rats is suppressed by pregnancy (T. Hamaguchi, unpublished data) and soy isoflavones [18].

In the present study, we established and characterized seven cell lines isolated from mammary tumors developed in a Tg rat. The *p53* tumor suppressor gene was mutated in only one cell line (C11), whereas the human (but not rat) *Ha-ras* genes were mutated in all seven cell lines. C11 cells, which overexpress the mutant p53 with a single amino acid substitution in the DNA-binding domain, were more sensitive to apoptosis triggered by DNA-damage than the other cell lines, which had wild-type p53. The mutant p53 may induce apoptosis in a transcription-independent manner in C11 cells.

2. Materials and methods

2.1. Establishment of carcinoma cell lines from DMBA-induced mammary tumors in a Tg rat and culture

Mammary carcinoma cells were isolated and cultured according to the method described by Hallowes et al. [21] with minor modifications. Briefly, 1.5 g of mammary tumor tissue was minced and incubated in 20 ml of 199 medium (Gibco/BRL, Grand Island, NY) containing 0.2% (300 u/ml) collagenase type I (Gibco/BRL, Grand Island, NY), 0.1% (460 u/ml) hyaluronidase (Gibco/BRL, Grand Island, NY) and 5% fetal calf serum (FCS) for 2 h at 37 °C. Dissociated cells were pelleted at 200 rpm for 5 min, resuspended in 20 ml of 199 medium, and sequentially passed through 500 μm and 70 μm filters (Becton Dickinson, Franklin Lakes, NJ) to remove large clumps. The filtrate was then poured over a 40 μm filter to collect the cell pellet by trapping it on the filter surface. Cells were incubated to induce attachment to culture dishes for 2 h in DMEM/F12 containing 5% FCS, 5 μg/ml insulin (Gibco/BRL, Grand Island, NY), 0.5 μg/ml hydrocortisone (Sigma, St Louis, MO) and 0.1 μg/ml progesterone (Sigma, St Louis, MO). The unattached epithelial cells were re-plated onto new dishes and incubated in the same medium. After establishment of cell lines derived from the single colonies by limiting dilution, another round of limiting dilution was performed to obtain the individual cell lines (C1, C2, C3, C6, C11, C15