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Interleukin-6 decreases senescence and increases telomerase activity in malignant human cholangiocytes

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Abstract

Background/aims: Cellular senescence results in irreversible growth arrest. In malignant cells, senescence is prevented by maintenance of chromosomal length by telomerase activity. Telomerase activity is increased in malignant, but not in normal cholangiocytes. Interleukin-6 (IL-6) is an autocrine promoter of cholangiocarcinoma growth. Our aims were to assess the relationship between IL-6 activated p38 mitogen-activated protein kinase (MAPK) pathways and senescence in malignant cholangiocytes.
Methods: Cell senescence and telomerase activity was assessed in Mz-ChA-1 malignant human cholangiocytes. The effect of inhibitors of p38 MAPK and telomerase activity on cell proliferation was assessed, and the interaction between these inhibitors was quantitated by median effects analysis.
Results: Mz-ChA-1 cells rapidly underwent senescence during repeated passaging. IL-6 increased telomerase activity and decreased cellular senescence during repeated passaging. However, basal telomerase activity was increased by inhibition of p38 MAPK. Inhibition of telomerase activity decreased IL-6 induced proliferation and had a synergistic effect with p38 MAPK inhibitors. Thus, IL-6 increases telomerase activity independent of p38 MAPK signaling and maintenance of telomerase activity promotes cholangiocarcinoma growth.
Conclusion: Enhanced telomerase activity in response to IL-6 stimulation can prevent cellular senescence and thereby contribute to cholangiocarcinoma growth. Inhibition of telomerase activity may therefore be therapeutically useful in biliary tract malignancies.

Keywords: Cholangiocarcinoma; p38 MAPK; Proliferation; Biliary tract; Cytokines

Introduction

Cellular senescence is a condition of irreversible growth arrest and serves as an important tumor suppressive mechanism by limiting cell growth (Reddel, 2000; Ishikawa, 2000). Replicative senescence occurs when normally dividing cells enter into a non-replicative state after undergoing a finite amount of cell division (Harley, 1991). Senescence is related to progressive shortening with each cell division of simple, tandemly repeated DNA sequences located at the ends of chromosomes termed telomeres (Variri and Benchimol, 1998; Bodnar et al., 1998; Karlseder et al., 2002). When the telomeric ends are either critically shortened or their function is disrupted, cells undergo senescence, which is characterized by cessation of cell division and replication. Telomerase is a multi-subunit ribonucleoprotein reverse transcriptase enzyme complex that serves to elongate the telomeric ends and compensate for the progressive shortening associated with DNA replication during each cycle of cell division (Greider and Blackburn, 1985; Blackburn, 1992). Thus, telomere shortening and senescence can be inhibited by maintenance of telomerase activity. Although it is repressed in most somatic tissues and non-malignant cells, telomerase is expressed in rapidly proliferating cells such as malignant cells, germ-line cells and stem cells (Broccoli et al., 1995; Meyerson, 2000; Kim et al., 1994). Telomerase expression in rapidly proliferating tumor cells maintains chromosomal length and prevents senescence (Variri and Benchimol, 1998; Bodnar et al., 1998; Greider, 1998).

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Tumors arising from biliary tract epithelia, or cholangiocarcinomas, are difficult to treat and are associated with a poor prognosis (Gores, 2003; Sirica, 2005). The dramatic increase in the global incidence and mortality from cholangiocarcinoma along with their unresponsiveness to conventional chemotherapeutic agents underscores the need for novel and improved treatments for this tumor (Patel, 2001, 2002). Telomerase activity is increased in malignant cholangiocytes, and is present in dysplastic cholangiocytes, but not in normal cholangiocytes (Iki et al., 1998; Itoi et al., 2000; Ozaki et al., 1999; Morales et al., 1998). Escape from senescence may be an important contributor to malignant transformation or tumor progression in biliary tract epithelia. However, the molecular mechanisms of telomerase regulation in human cholangiocarcinoma are unknown.

The inflammatory cytokine interleukin-6 (IL-6) is mitogenic for biliary tract epithelia and promotes growth of human cholangiocarcinoma (Matsumoto et al., 1994; Yokomuro et al., 2000; Park et al., 1999a,b; Okada et al., 1994). We and others have shown that IL-6 can act as an autocrine factor and promote proliferation of malignant cholangiocytes. Proliferation in response to IL-6 involves activation of the p38 mitogen-activated protein kinase (MAPK) signaling pathways in malignant but not in non-malignant cholangiocytes (Tadlock and Patel, 2001; Park et al., 1999b). p38 MAPK signaling mediates a transformed cell phenotype in malignant cholangiocytes manifest by decreased serum dependency, anchorage independent growth and enhanced xenograft growth in nude mice. Despite the close association between telomerase expression and cellular proliferative capacity, the relationship between mitogenic signaling and regulation of telomerase activity in malignant cholangiocytes is unknown. Recent studies suggest that premature senescence represents a cellular mechanism of chemoresistance suggesting that strategies to prevent senescence may improve therapeutic responses (Schmitt et al., 2002; Te Poele et al., 2002). Thus, the overall aim of our study was to investigate the effect of the biliary epithelial mitogen IL-6 on telomerase activity and to examine the relationship between maintenance of telomerase activity and IL-6 induced cell proliferation in malignant human cholangiocytes. We addressed the following questions in our study: Does stimulation by IL-6 alter cellular senescence or telomerase activity? Is regulation of telomerase activity dependent on p38 MAPK signaling? If so, is p38 MAPK signaling involved in modulation of telomerase activity by IL-6? Does inhibition of telomerase activity modulate mitogenic effect of IL- 6?

Materials and methods

Cells

Mz-ChA-1 cells, derived from metastatic gall bladder cancer, were provided by Dr. J.G. Fitz (University of Texas Southwestern, Dallas, TX) and cultured in CMRL 1066 media with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin (100 IU/ml)/streptomycin (100 mg/ml) mix. Cells were used at 50–60% confluency, washed twice with phosphate-buffered saline (PBS) and incubated in serum-free media for 24 h prior to study. KMCH, malignant intrahepatic cholangiocytes, and H69 cells, non-malignant intrahepatic cholangiocytes, were obtained and cultured as previously reported (Park et al., 1999b). For MAPK or telomerase inhibitor studies, cells were pre-incubated with the inhibitors for 1 h prior to the experiments.

Generation of stably transfected cell lines

Mz-ChA-1 cells were stably transfected with an expression plasmid containing full-length IL-6 under the control of a CMV promoter and designated as Mz-IL-6. Control cells were transfected with empty vector. Plasmids were purified using the Qiagen Plasmid Midi Kit (Qiagen Inc., Valencia, CA) and linearized by restriction enzyme digestion prior to transfection using Trans-IT (Panvera, Madison, WI). A mixed population of stable transfectants was obtained by selection in media containing G418 for 3 weeks. Stable transfection was confirmed using an IL-6 bioassay to verify IL-6 over-expression. Basal expression of IL-6 was increased two- to three-fold in Mz-IL-6 cells relative to controls. For studies of p38 MAPK signaling pathways, Mz-p38DN cells were derived by stable transfection of Mz-ChA-1 cells with an inactive upstream activator of p38 MAPK signaling MKK3(A). These cells demonstrate decreased constitutive expression of p38 MAPK and were generated as we have recently described for KMCH cells (Yamagiwa et al., 2003).

Senescence assay

Cellular senescence was detected by staining for senescence associated acidic β-galactosidase (SA-β-gal) (Dimri et al., 1995; Bodnar et al., 1998). Cells were fixed with 2% formaldehyde and 0.2% glutaraldehyde in PBS and incubated for 24 h at 37 °C with β-gal staining solution containing 1 mg/ml X-gal, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl, 2 mM $MgCl_2$, 40 mM citric acid with Na_2HPO_4 (pH 6.0). The proportion of senescent cells at each time point was assessed by counting the percentage of SA-β-gal positive cells in at least 2×10^3 total cells using light microscopy.

Cell proliferation

Cells were seeded into 96-well plates (10,000 cells/well) and incubated in a final volume of 200 μl medium. The cell proliferation index was assessed as we have previously described using a commercially available colorimetric assay (CellTiter 96AQueous, Promega Corp., Madison, WI) (Park et al., 1999b).

Telomerase assay

Telomerase activity in cell extracts was measured by the PCR-based telomere repeat amplification protocol (TRAP) using the TRAPeze kit (Intergen, Gathiesburg, MD). Briefly, the cells were grown in 6-well plates, washed in PBS, and