Background and Objective

Chondrosarcoma are poorly understood malignant cartilage neoplasms that vary considerably in biologic behavior. Resection provides fair outcomes, and neither chemotherapy nor radiation therapy is efficacious. Limited biological literature now exists to form a basis for advancing therapy. The anticancer tumor suppressor transcription factor, p16, and p53, have an established role in many cancers, responding to a variety of cellular stresses, including DNA damage and oncogene activation (1,3,4). In chondrosarcoma, the p16/pRB/cdk4 pathway, rather than the p53 pathway, has been suggested to play the pathogenic role (5). The p16 tumor suppressor protein, which inhibits cyclin-dependent kinase 4 and cyclin-dependent kinase 6, is implicated in inciting replicative senescence, the state of permanent cellular growth arrest (4). Clarifying the molecular pathways that chondrosarcoma exploit to avoid senescence growth arrest, and to survive genomic crisis, the key steps preceding immortality, may reveal intervention points for better diagnosis, prognosis, or treatment (1).

Our previous work with an in-vitro model of human chondrosarcoma malignant transformation has correlated p16 inactivity and telomerase activity with: a) accelerated proliferation; b) survival of growth arrest; and c) immortality (2). We hypothesize that malignant transformation in chondrosarcoma depends on successful transduction through replicative crisis. With crisis, comes severe chromosomal derangement (7), and global gene expression changes associated with malignant transformation.

Our objective is to induce expression of the p16 tumor suppressor in CS-8 cells at different points in their growth phase (both in early passage p16+ cells and late passage p16- cells) and assess effects on population doubling, replicative growth arrest, senescence, and apoptosis. We hypothesize that in malignant late passage CS-8 cells (p16−, telomerase +), p16 may spur replicative growth arrest by restoring senescence signaling, or suppress the emergence of proliferating transformed cells.

Methods

Our CS-8 line was isolated from a slow-growing grade I human chondrosarcoma and cultured as described previously(2). We have shown that early passage CS-8 are both telomerase− and p16+, but that with in-vitro survival beyond growth arrest, the late-passage (post-crisis) CS-8 telomerase+, and p16− (2), as shown by TRAP for telomerase and Westerns for p16, respectively (2).

To transfer p16 into CS-8, we choose the tetracycline-controlled transactivator responsive promoter (Tet) retroviral gene transfer system (Clontech) for inducible control of p16 expression. A full-length cDNA clone encoding p16 in the vector pUHDD10-3 (gift from Dr. Bujard in Heidelberg) was subcloned in pBS sk- at the EcoR1 site. A BamH1 and HindIII digested fragment was then cloned in pRev-TRE (Figure 1).

Figure 1. pRevTRE+p16 plasmid

Recombinant packaging cell lines (RPMI) was transfected with the retroviral plasmids, and the medium was then harvested and filtered. CS-8 were transduced with retrovirus carrying p16. The transduced and control CS-8 cells will be analyzed. At this point, we anticipate that the effect of p16 induction on late passage CS-8 population growth curves will be either to trigger or to promote permanent growth arrest (senescence) or both (Figure 2). Absence of p16 could also serve as a prognostic marker of the potential for chondrosarcoma aggressiveness, as has been suggested for lung cancer (6), although further pathologic-clinical correlation is needed. Regarding therapy, the potential for inducible retroviral vector gene therapy for cancer is not yet fully clear in terms of feasibility and efficacy, but the use of tumor suppressor gene therapy as a part of multimodal chondrosarcoma therapy may be reasonable.

Figure 2. Effects of p16 Transduction on CS-8 Growth

We use beta-galactosidase staining as a senescence marker, as previously described (1,3), and annexin-V/propidium iodide staining (Invitrogen) via flow cytometry as an apoptosis marker.

DNA population doubling (PD) growth curves will be contrasted among late passage CS-8 cells, early passage CS-8 cells, and all p16 transduced CS-8 cells. Mixed-model ANOVA’s will allow contrasting of the p53 cell populations to best characterize the timing of the escape from the growth plateau (either positive escape [control], or negative escape [apoptosis], or lack of escape [senescence]) (Figure 2).

Essential Results and Discussion

Preliminary work has used PCR to confirm the presence of inducible p16 with doxycycline (100 ng/ml) in the RevTet-On/RevTRE-p16 transduced late-passage CS-8. This will be confirmed and correlated with p16 Western analysis, as previously described (2). Senescence, apoptosis, and population doubling growth curves of the transduced and control CS-8 cells will be analyzed. At this point, we anticipate that the effect of p16 induction on late passage CS-8 population growth curves will be either to trigger or to promote permanent growth arrest (senescence) or both (Figure 2).

References