Thus, even though the D16S3136 p values we obtained did not reach statistical significance, they are of interest because they provide limited evidence for possible linkage disequilibrium at this locus. In evaluating these data, it is important to consider that only a limited subset of psoriatic patients may carry disease-predisposing NOD2 variants (i.e., genetic heterogeneity) and that multiple allelic variants of NOD2 might predispose to psoriasis (i.e., allelic heterogeneity). Either of these situations would be expected to weaken the statistical evidence for linkage disequilibrium. Additional, confirmatory studies will be required in order to determine whether the clinical concomitance of psoriasis and CD is due to the existence of one or more disease alleles at NOD2.

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 XP43TO, Previously Classified as Xeroderma Pigmentosum Group E, Should be Reclassified as Xeroderma Pigmentosum Variant

To the Editor

Xeroderma pigmentosum (XP), a rare autosomal recessive disease characterized by clinical and cellular hypersensitivity to ultraviolet (UV) light (Cleaver and Kraemer, 1995), has been classified into eight genetic groups [A–G, and variant (V)]. XP-E (OMIM no. 278740) was reported to have biochemical heterogeneity regarding a damage specific DNA binding (DDB) activity (Kataoka and Fujiwara, 1991; Keeney et al, 1992); some strains lacked DDB activity and were termed Ddb− XP-E, whereas others had activity and were termed Ddb+ XP-E. After reinvestigating three Ddb+ XP-E cell strains, we recently reported that each had been misclassified, and based on their phenotypes after UV-irradiation (unscheduled DNA synthesis, recovery of RNA synthesis, and recovery of replicative DNA synthesis in the presence and absence of caffeine) we tentatively reclassified them as XP-E (OMIM no. 278760, XP89TO), XP-V (OMIM no. 278750, XP43TO), and ultraviolet-sensitive syndrome (OMIM no. 600630, XP24KO) strains (Itoh et al, 2000a). We therefore supported the tentative proposal of Cleaver et al (1999) that mutations in the DDB2 gene should be solely responsible for XP-E (Itoh et al, 2000a; Nichols et al, 2000).

As the assignment of XP43TO as XP-V was based upon the recovery of replicative DNA synthesis in the presence of caffeine, we have analyzed the XP43TO for mutations in the XPF gene. The XPF gene codes for DNA polymerase η (Johnson et al, 1999; Masutani et al, 1999), an inducible, damage bypass DNA polymerase (Yamada et al, 2000). RT-PCR products from base pairs 1130–2410 of the XPF gene were cloned into a broad band compared with the products from normal TUR cells (RT-PCR1, Fig 1A). This broadening was not seen, however, for products from base pairs 898–2416 (RT-PCR2, Fig 1A).


One clones from the XP43TO RT-PCR1 band were sequenced (Fig 1b). Twelve of these contained one of four types of exon omission, whereas 19 contained one of three types of abnormal splicing events around exon 4. Moreover, exon 4 contained a G→T transversion at nucleotide 727 that would generate an E164ter nonsense mutation (Fig 1b). By contrast, six independent clones of this region from normal Turu cells had the sequence reported by Masutani et al (1999) and Johnson et al (1999).

To verify that XP43TO cells carry this mutation in genomic DNA, we performed PCR with genomic DNA and direct sequencing of the region between exon 4 and intron 4 and confirmed the presence of the G→T transversion (Fig 2a). Significantly, nucleotide 727 is the highly conserved G(±1) at the splice donor site of exon 4 (Fig 2b). These results confirm that the patient is either homozygous for the transversion or contained a large deletion of the region in the other allele. In either case, XP43TO should be classified as XP-V.

Of the types of XP43TO cDNA found (Fig 1b), types 3, 4, and 5 resulted in frameshifts and type 7 had an in-frame stop codon in the sequence inserted from intron 4. Types 1 and 2 would produce a protein deleted for all of the seven DNA polymerase conserved motifs as identified by Yamada et al (2000) and Kannouche et al (2001). Type 6 would give rise to a 30 amino-acid deletion between the fourth and fifth motif that could compromise the function or stability of the protein. This large distribution of mRNA types could be due to the transversion, or XP43TO could harbor one or more mutations in the early introns, that could also give rise to splice variants. Alternatively, some or all of the alternative splicing events might occur at a low level in normal cells, but the PCR might not be sensitive enough to detect them in the Turu cells. That is, the distribution might include some minor alternatively spliced forms present in normal cells.

We currently favor the hypothesis that all strains originally reported as Ddb+ XP-E by cell fusion were originally misclassified; however, strict proof of this proposal is not possible because some of these strains have been lost and others are available only as late passage samples for which phenotypic analysis is not reliable.
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A Simple In Vivo System for Studying Epithelialization, Hair Follicle Formation, and Invasion Using Primary Epidermal Cells from Wild-Type and Transgenic Ornithine Decarboxylase-Overexpressing Mouse Skin

To the Editor:

We report here a simple system for studying epithelialization, hair follicle formation, and tumorigenesis by epidermal keratinocytes. We have adapted a tracheal xenotransplantation assay originally developed to evaluate invasiveness of human and mouse bronchial epithelial cell lines (Terzaghi et al, 1978; Momiki et al, 1991). This technique involves the seeding of epithelial cells into deepithelialized tracheal grafts that are then transplanted subcutaneously into athymic nude mice. Both nontumorigenic and premalignant epithelial cell lines reepithelialize these tracheas within a few weeks by growing on the luminal surface of the tracheal grafts (Smith et al, 1997). Stromal cells are contributed by the host mouse, and result in the repopulation of cells and blood vessels in the lamina propria of the tracheal grafts. Thus, tumor cell invasiveness that is dependent on cell–matrix interactions can be fully evaluated using this tracheal xenotransplantation assay. Momiki and Klein-Szanto have classified the degree of invasion of the tracheal wall according to the level of penetration of the cells being tested, with level 0 describing transplanted cells confined to the lining of the luminal surface, level 2 involving infiltration of the lamina propria, the pars membranacea, and the trachealis muscle but not the adventitia, and level 3 for when the malignant cells have reached the adventitia and the whole tracheal wall is invaded (Momiki et al, 1991). This method offers several advantages over in vitro assays in that it more closely simulates the in vivo conditions in which epithelial tumor cells invade neighboring tissue. Moreover, the cells under evaluation are contained in a small, defined area of the tracheal graft that also serves to separate the tested epithelial cells from endogenous epithelial cells of the host athymic nude mouse. Infiltration of grafted cultures of epithelial cells by host epithelial cells surrounding the graft site can be a problem with some grafting procedures such as skin reconstitution methods. In addition, far fewer cells are needed to re-epithelialize the lumen of a tracheal graft than are needed in other epithelial grafting procedures, thus offering the potential to study stem cells derived from the cutaneous epithelium where the number of inoculating cells is limiting. We have tested the feasibility of growing primary cultures of murine keratinocytes in tracheal xenotransplants in order to develop a simple, enclosed system in which epidermal cells can be evaluated in vitro.

INVASION ASSAY

We used the tracheal xenotransplantation procedure to evaluate invasive properties of primary cultures of keratinocytes that express elevated levels of ODC and/or v-Ha-ras. As a source of cells that express high levels of ODC, we took advantage of the K6/ODC transgenic mouse in which a keratin 6 promoter is used to target ODC from the skin of these newborn animals are cultured, resulting in high levels of ODC activity in the cultured keratinocytes. Half of the cultured keratinocytes were infected with a replication-defective v-Ha-ras retrovirus (Roop et al, 1986), and then inoculated 2–3 d later in prepared deepithelialized rat tracheas with a blunt-tipped needle at a concentration of 10^7 cells per ml (0.5x0–0.125 ml per trachea depending on tracheal size). The inoculated tracheas were sealed at the ends with hemoclips, stretched onto thin Teflon tubing, and implanted subcutaneously into athymic mice for 5 wk to assay the degree of invasion of the...