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Drug-Induced Alterations of Cytokeratin Organization in Cultured Epithelial Cells

Abstract. *The distribution of keratin intermediate filaments, previously considered static in organization and imperturbable by conventional drugs used to alter the structure and organization of the cytoskeleton, can be altered significantly by treatment with colchicine and cytochalasin D. The loss of microfilaments and microtubules converts the keratin cytoskeleton from a branching, even distribution to a series of starlike structures whose filaments are maintained by multiple membrane attachment sites. These findings provide a means for manipulating cytokeratin organization to investigate the role of keratins in cytoskeletal structure and function.*

Although most types of intermediate filaments (IF's) are known to be structural components of the vertebrate cytoskeleton, their role in the function of the cytoskeleton has yet to be established. Immunological and biochemical evidence has revealed at least five distinct classes of IF's: keratin filaments, vimentin filaments, desmin filaments, glial filaments, and neurofilaments (*1–3*). Defining the structural and functional relations among IF's and between IF's and the two other major cytoskeletal filament systems, microtubules and microfilaments, is an important area of research in cell biology. Keratins are of particular interest in studying these relations because they (i) are found as cytoskeletal elements (cytokeratins) in virtually all higher vertebrate epithelial cell types both in vitro and in vivo (*4–7*); (ii) represent a system of tonofilaments whose organization is characterized in numerous epithelia (*8–12*); (iii) are found in tumor cells of epithelial origin (*1, 13*); and (iv) are the major products of differentiation for the epidermal cells of the integument (*14*).

We report that the distribution of cytokeratin IF's can be significantly altered by treatment with a combination of colchicine and cytochalasin D. Cytokeratins in transformed and fetal mouse epidermal cells undergo a rapid transition from an even distribution (Fig. 1A) to a lattice of keratin fibers interconnecting membrane-associated focal centers (Fig. 1, B and C).

Keratin filaments were identified by using indirect immunofluorescence microscopy with antiserum to α-keratin isolated from avian stratum corneum (*15*). Rabbit antiserum to chicken α-keratin, like antiserums to mammalian α-keratins, cross-reacts with keratins from other higher vertebrates, including mice. Stably transformed cultures of the KLN 205 clone of a squamous cell carcinoma (*16*) derived from a DBA/2 mouse tumor were grown on glass cover slips and maintained in Leibovitz-15 medium supplemented with 10 percent fetal bovine serum. Primary cultures of DBA/2J mouse epidermal cells from 14-day fetuses were grown under the same culture conditions.

Both cell types were treated for 2 hours at 37°C with cytochalasin D (0.5 μg/ml) in 1 percent dimethyl sulfoxide (DMSO), colchicine (40 μg/ml), or a combination of the two. Controls included untreated cells, cells treated with 1 to 10 percent DMSO, and cells treated with colchicine and 1 percent DMSO. The cells were fixed in methanol and made permeable with acetone for 5 minutes each at −20°C and air-dried before being processed for immunofluorescence. Indirect immunofluorescence with preabsorbed rabbit antiserum to chicken α-keratin or with serum from unimmunized rabbits demonstrated no fluorescence. Control cells and cells treated with cytochalasin D or colchicine alone displayed a normal cytoplasmic distribution of keratin filaments (Fig. 1A).

Cytochalasins and colchicine derivatives have been used to study the roles of microfilaments and microtubules, respectively, in cell morphology, motility, and division (*17–20*). These antimitotic drugs have been tested for indirect effects on the various IF systems with limited success (*7, 21*). Only an effect by Colcemid on vimentin filament distribution has been reported (*21*). Neither Colcemid nor cytochalasin B alone elicits any change in the organization of keratin filaments (*7, 21*). However, we have induced rearrangement of cytokeratins by disrupting the integrity of microtubules and microfilaments simultaneously. The depolymerizing effects of colchicine and cytochalasin D have been confirmed in both KLN 205 carcinoma cells and fetal mouse epidermal cells by indirect immunofluorescence with antiserum to actin or tubulin. Cytochalasin D elicits extensive surface retraction of spread epithelial cells. As a result, the margins of the cells retain contacts with other cells only at focal adhesive sites similar to those seen between KLN 205 cells (Fig. 1D). However, the organization of keratins throughout the cytoplasm is generally undistrubed unless both cytochalasin D and colchicine are used (Fig. 1A).

The effect of the combination of colchicine and cytochalasin D on the organization of cytokeratins suggests some kind of direct interaction between keratin filaments and microtubules and microfilaments. Alternatively, changes in

cell morphology or cytoplasmic structure induced by a loss of microtubules and microfilaments may result indirectly in compensatory restructuring of the keratin cytoskeleton. In addition to the lattice-like cytokeratin filament network itself, the cells have membrane-associated sites with which the keratin filaments interact (Fig. 1C). While these sites are not observed in undisturbed cells (Fig. 1A), the loss of microtubules and microfilaments converts the keratin cytoskeleton into a series of starlike structures that are clearly organized and perhaps prevented from collapsing by the membrane-associated sites that punctuate the surface of the cells (Fig. 1B). Filament attachment sites may also occur in the cytoplasm, unassociated with the cell membrane, but this remains to be established.

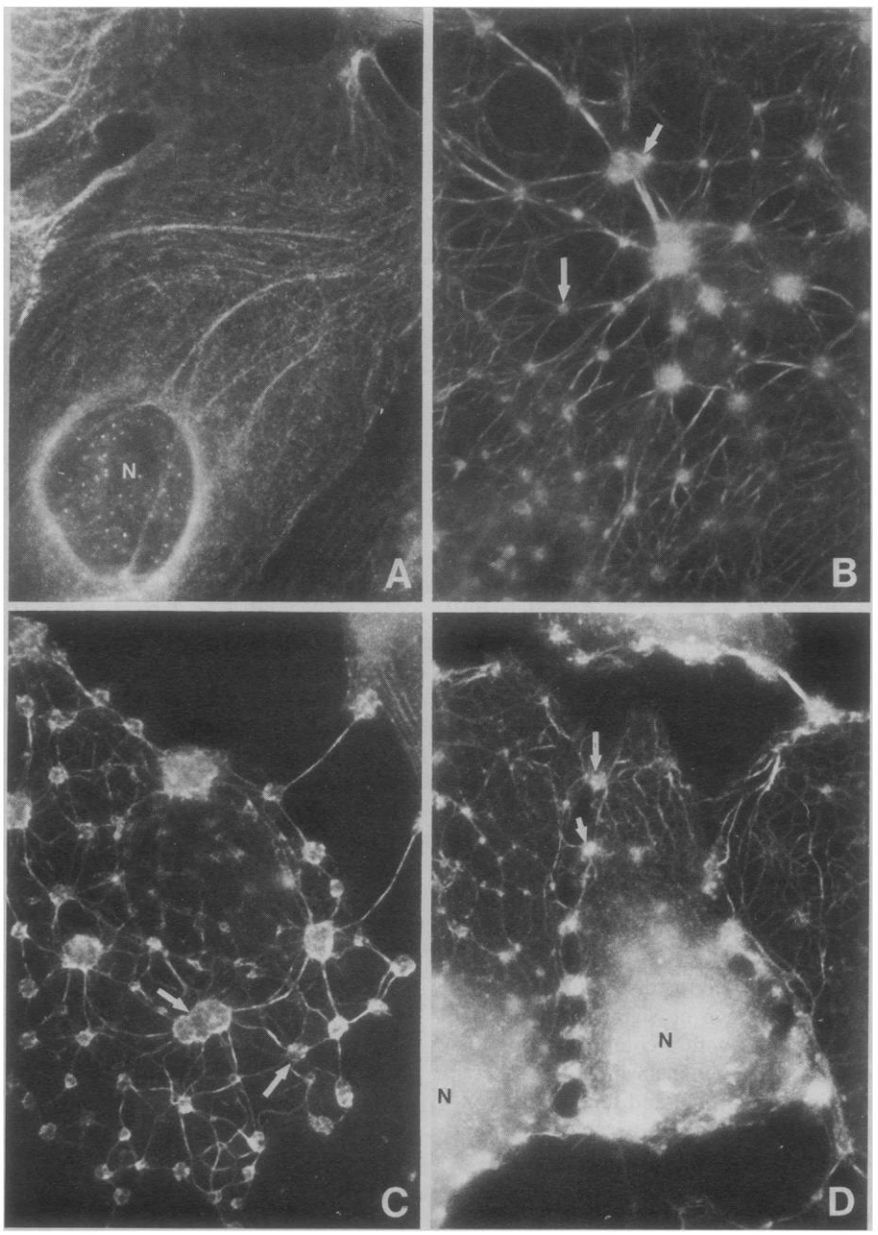

Fig. 1. (A) Mouse KLN 205 carcinoma cell from a control culture treated with 1 percent DMSO. Keratin filaments form an evenly distributed branching network typical of all control cell cultures and of cultures treated with cytochalasin D or colchicine. The unstained nucleus (*N*) is surrounded by cytokeratin filaments (×1500). (B) Mouse KLN 205 carcinoma cell treated for 2 hours with colchicine and cytochalasin D in DMSO. Cytokeratin filaments in the area shown and throughout the rest of the cell form a lattice that is maintained by focal centers (arrows). The keratin filament–focal center complexes form starlike structures throughout the cytoplasm (×1500). (C) DBA/2J mouse epidermal cell treated for 2 hours with colchicine and cytochalasin D in DMSO. Focal centers maintain the system of cytokeratin filaments. Large centers (arrow) may result from fusion or overlap of two or more centers (×1500). (D) Mouse KLN 205 carcinoma cells treated for 2 hours with colchicine and cytochalasin D in DMSO. Three adjacent cells are shown, connected by intercellular junctions. Cytokeratin filaments in the cells converge at these sites (arrows). The nuclei are out of the focal plane (×600).

The change in the organization of cytokeratins induced by colchicine and cytochalasin D is relatively rapid. This contrasts with the long-term treatment with Colcemid required to disrupt vimentin filament organization. Treatment of cultured cell lines with Colcemid results in extensive redistribution of the vimentin filaments into a poorly organized cytoplasmic cap or perinuclear whorl (*21*). Keratin filaments, on the other hand, maintain an independent network organized by a system of membrane plaques (Fig. 1B). Regardless of their final organization or lack of organization, keratin and vimentin filaments apparently associate with or are fundamentally affected by the presence or organization of other cytoskeletal elements.

It is thought that *desmosomes* and *hemidesmosomes* act as organization centers for cytokeratin filaments (*4, 8, 10–12, 22*). Our results support this contention. The filaments radiate from and are maintained by multiple membrane attachment sites. This starlike array of keratin filaments emanates from basal cell surface hemidesmosomes or substratum attachment plaques. In addition, a periodic distribution of attachment sites occurs between apposed cells (Fig. 1D). This pattern of intercellular connections is further evidence that desmosomes play a major role in the organization of cytokeratin filaments. The distribution of these structures corresponds to the distribution reported by Franke *et al.* (*23*) for basal cell surface desmosomal proteins in cultured epithelial cells and correlates with fine structural evidence showing tonofilament associations with desmosomes (*8–12*).

The capability to alter the organization of the keratin cytoskeleton in epithelial cells by treatment with specific antimitotic drugs provides a new means to investigate the role of keratins in cytoskeletal function and organization. Further characterization of the interactions between keratins and other elements of the cytoskeleton and between keratin filaments and membrane-associated organization centers will increase our understanding of subcellular structure and function.

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5′ Viral and Human Cellular Sequences Corresponding to the Transforming Gene of Simian Sarcoma Virus

Abstract. *The 5′ nucleotide sequences of the transforming gene of simian sarcoma virus (v-*sis*) and its human cellular homolog (c-*sis*) were compared. A short homology was found between helper virus and cellular DNA sequences at the junction of v-*sis *and c-*sis*, which may have had a role in the original recombination event leading to the generation of simian sarcoma virus.*

Retroviruses are etiological agents in naturally occurring leukemias and lymphomas in a number of animal species (*1*). Most of these viruses cause disease after long latency periods, and their genomes do not contain a definable transforming gene. In contrast, the rapidly transforming retroviruses contain transforming genes (v-*onc* genes) as a result of recombination with normal cellular (c-*onc*) genes (*2*). The latter are highly conserved and may be important for cell growth or differentiation (or both). The simian sarcoma virus (SSV), a transforming virus isolated from a spontaneous fibrosarcoma of a pet woolly monkey (*3*) contains a 1.0-kilobase (kb) transforming gene (v-*sis*) that is derived from woolly monkey cellular DNA sequences (*4–7*). The helper virus (SSAV) associated with SSV is highly homologous to the gibbon ape leukemia viruses (GaLV's) (*8*). Thus, an infection of the woolly monkey by a virus of the GaLV group probably preceded the generation of SSV.

In the human genome, *sis*-related sequences are present at a single locus, which is interrupted by at least four stretches of nonhomologous cellular sequences (*9, 10*). A 4.2-kb c-*sis* transcript is detected in some human tumor tissues (*11, 12*), suggesting that the functional c-*sis* gene contains coding sequences additional to the 1.0-kb v-*sis* gene. The exact relation of the viral to the cellular gene homolog and the molecular basis of the mechanism by which SSV and other *onc*-containing retroviruses are generated are not known, although several possibilities have been proposed (*13, 14*). As a step toward understanding such mechanisms, we have compared the nucleotide sequences at the 5′ recombination site of SSV to the 5′ analogous region of the related human cellular homolog, c-*sis*. We found a 6-base-pair (bp) sequence homology between helper virus and cellular DNA sequences immediately preceding the v-*sis* and c-*sis* regions of homology. This 6-bp homology may have played an important role in the recombination process that led to the generation of SSV.

The complete nucleotide sequence of v-*sis* has been determined [(*15*) and our unpublished data]. The results showed that the v-*sis* gene potentially codes for a 27,000-dalton protein that is initiated within the helper-derived sequences and is terminated at a site two-thirds within the v-*sis* substitution. To understand better the 5′ boundary of the cellular *sis* gene, we determined a portion of the nucleotide sequence of a human c-*sis* gene. The structure of a recombinant DNA, L33, containing 12 kb of human DNA, including the entire 1.0 kb of v-*sis* homologous sequences, is shown in Fig. 1. The five noncontiguous regions of homology, designated as black boxes numbered 1 to 5, are colinear with v-*sis*, as determined by heteroduplex analyses and restriction enzyme mapping (*10*). The 5′ Eco RI–Pst I fragment was subcloned into the plasmid pBR322 (*16*), and a 596-nucleotide sequence, including the first region of homology and surrounding sequences, was determined by the Maxam-Gilbert procedure (*17*) (Fig. 1). This nucleotide sequence (Fig. 2A) re-

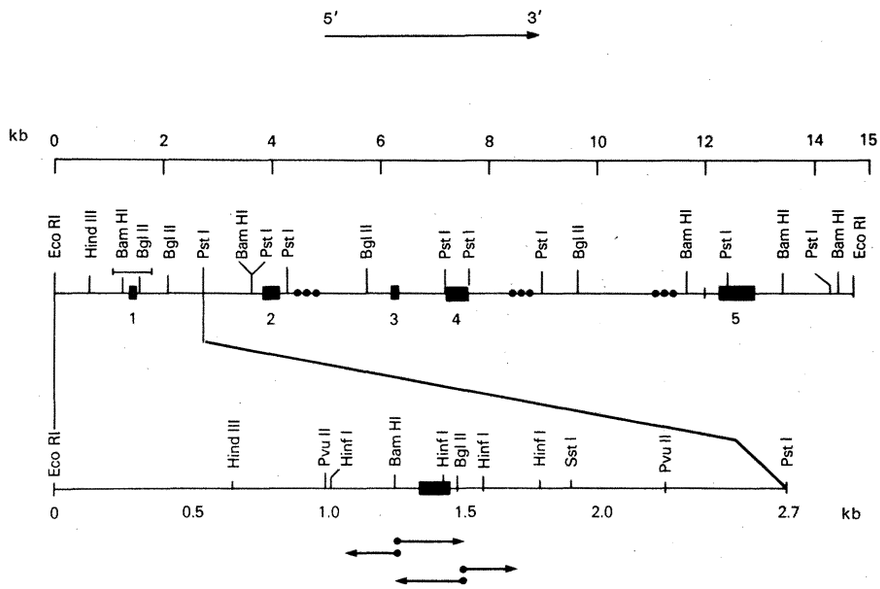

Fig. 1. Genetic structure of a human DNA clone (L33) containing sequences homologous to v-*sis*. The black boxes numbered 1 to 5 represent the noncontiguous regions of homology with v-*sis*. The dots show the location of the Alu family of repeated sequences (*10*). Arrows under the expanded map of the 5′ Eco RI–Pst I fragment indicate the directions for DNA sequencing by the Maxam-Gilbert procedure (*17*).