SUPPRESSION OF CHONDROGENIC EXPRESSION IN MIXTURES OF NORMAL CHONDROCYTES AND BUDR-ALTERED CHONDROCYTES GROWN IN VITRO

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Abstract:

When normal chondrocytes are mixed with suspended liver, or kidney, or muscle cells and reared in monolayers in vitro, the heterotypic cells suppress the synthesis of chondroitin sulfate by adjacent chondrocytes. Chondrocytes grown in BudR, though replicating normally, do not synthesize chondroitin sulfate. BudR-altered chondrocytes exhibit cell surface properties very different from those exhibited by functional chondrocytes. BudR-altered cells do not adhere to one another and they are amoeboid. In mixtures of BudR-altered chondrocytes and normal chondrocytes, the former cells function as heterotypic cells: BudR-altered cells suppress chondroitin sulfate synthesis by contiguous normal chondrocytes.

In organ cultures suspended chondrocytes mixed with suspended liver or kidney cells segregate according to cell type (Townes and Holtfreter, '55; Moscona, '61; Steinberg, '63). In contrast, when suspended chondrocytes are mixed with liver or kidney cells and grown in monolayer cultures, the chondrocytes become amoeboid and fail to deposit matrix (Abbott and Holtzer, '64). This interaction whereby the proximity of cells of one phenotype blocks the expression of cells of another phenotype has been termed

"interference." It is postulated that proximity of heterotypic cell surfaces or their exudates suppresses the synthesis of chondroitin sulfate by adjacent normal chondrocytes.

Chondrocytes in vitro form epithelioid colonies of non-motile, polygonal cells which adhere to one another (Coon, '66; Coon and Cahn, '66). They deposit metachromatic matrix, synthesize chondroitin sulfate, and sulfokinase and UDPGNA-4-epimerase activity is detectable. Chondrocytes grown in 5-Bromodeoxyuridine (BudR) and with BU in their DNA, replicate at the rate of untreated chondrocytes but repel each other, become amoeboid, and adhere to surfaces which do not bind normal chondrocytes. BudR-altered chondrocytes do not synthesize significant amounts of chondroitin sulfate, and do not have detectable levels of sulfokinase or UDPGNA-4-epimerase (Holtzer and Abbott, '68).

The experiments to be described were designed to answer the following: (1) In mixtures of normal and BudR-altered chondrocytes will the latter suppress the synthesis of chondroitin sulfate by adjacent normal chondrocytes? and (2) If the synthesis of chondroitin sulfate by normal chondrocytes is blocked by proximity to BudR-altered chondrocytes, do they yield normal progeny when removed from the cell-cell inhibitory action of BudR-altered cells?

Material and Methods: Chondrocytes were liberated from vertebral cartilages from 10-day chick embryos and floaters were cloned (10^4 cells/60mm petri dish) in double strength F-10 (Ham, '65) as described in Abbott and Holtzer ('68). BudR (5µg/ml) was added to half the dishes when the cultures were 4 days old. Untreated chondrocytes and BudR-treated chondrocytes were cultured for another 7 days. At this time, over 95% of all cells in the untreated cultures were polygonal, whereas all cells in the treated

cultures were fibroblastic. The cultures of untreated and BudR-treated cells were rendered into cell suspensions by treatment with trypsin, and used to establish the following series of second-generation cultures: (1) 4×10^5 BudR-altered cells mixed with 2×10^5 normal cells; (2) 4×10^5 BudR-altered cells; (3) 6×10^5 BudR-altered cells; (4) 2×10^5 normal cells; (5) 6×10^5 normal cells. The second-generation cultures were fed daily with normal medium, inspected under the phase microscope, and sacrificed after 8 days. Selected cultures were fixed and stained with toluidine blue for metachromatic polysaccharide matrix. Other cultures were incubated with $^{35}SO_4^=$ (Sodium salt, carrier-free, Abbott Lab.) for 24 hours during the 7th or 8th day of culture and the cells extracted for chondroitin sulfate by procedures described in Nameroff and Holtzer ('67). Total amounts of DNA were determined by the method of Burton ('56).

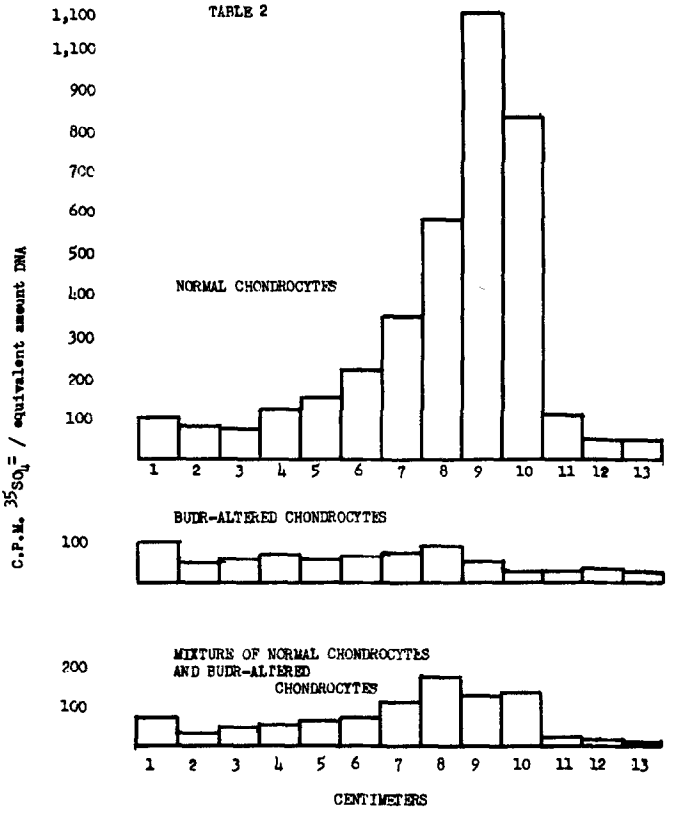
Results: As shown in Table 1, the progeny of chondrocytes in 8-day old second-generation cultures established from untreated cells are predominantly polygonal and extracellular metachromatic matrix surrounds many of the cells. The progeny of chondrocytes in similarly aged cultures established from BudR-treated parental cells are predominantly fibroblastic, although they have replicated in normal medium for 8 days. Metachromatic matrix is not present in these cultures. In the mixed cultures, small clusters (less than 10 cells) of polygonal, or fibroblastic cells can be observed attached to the plastic petri dishes on the first day. From days 2 to 8, however, the only cell type observed in these mixed cultures are fibroblastic or spindle-shaped. The cells in the mixed cultures either proliferate for a longer time or have a higher survival rate, for at the end of 8 days there are approximately twice the number of cells in the mixed population as in either of the control,

TABLE 1. Behavior of normal chondrocytes, BudR-altered chondrocytes, and mixtures of BudR-altered and normal chondrocytes in second-generation cultures

	4×10^5 BudR-altered plus 2×10^5 Normal cells	4×10^5 BudR-altered cells	6×10^5 BudR-altered cells	2×10^5 Normal cells	6×10^5 Normal cells
Over 85% cells Polygonal	-	-	-	+	+
Over 95% cells Fibroblastic	+	+	+	-	-
Metachromatic matrix	-	-	-	+	+

parental cultures. Eight-day old cultures do not reveal extracellular metachromatic polysaccharide. As shown in Table 2, labeled chondroitin sulfate is recovered from 7 or 8 day-old cultures of normal chondrocytes grown in $^{35}SO_4^{=}$ for 24 hours. If chondroitin sulfate is synthesized in cultures of BudR-altered cells, or in cultures of mixed BudR-altered and normal cells, it is approximately 10% of that synthesized by normal cells on a DNA basis.

To determine whether the progeny of normal chondrocytes in mixed cultures would rapidly assert their capacity to synthesize chondroitin sulfate when removed from the exogenous inhibitory influence of neighboring BudR-altered cells, the following experiment was performed: 6-day old cultures of second-generation



mixtures of BudR-altered and normal cells were rendered into a cell suspension and replated as 3rd generation cultures at low density (10^4 cells/dish). After 5 days the third-generation cultures exhibit many epitheliod colonies of polygonal cells, as well as loose colonies of fibroblastic cells. Metachromatic matrix is deposited around many of the polygonal cells and the cells incorporate labeled $SO_4^=$ into chondroitin sulfate. Presumably, separating normal chondrocytes from the local interfering effect of BudR-altered cells permits many of the former cells to replicate and express their chondrogenic phenotype.

Conclusion: It has been proposed that the suppressive effect of BudR on chondrogenesis (Abbott and Holtzer, '68; Coleman, et al, '68), on myogenesis (Okazaki and Holtzer, '66; Coleman, et al, '68) and on hyaluronic acid synthesis (Bischoff and Holtzer, '67) may be due to the BU-DNA leading to changes in properties of the surface of the cell. Proximity to the surfaces, or exudates of kidney or liver cells or BudR-altered chondrocytes, interferes with the characteristic synthetic activity of genetically programed chondrocytes. Inductive interactions between heterotypic (Fleischmajer and Billingham, '68) or homotypic cells during embryogenesis have often been described. Inhibitory interactions of the kind described in this report may operate in such inductive systems, as well as in other cell-cell or cell-matrix reactions (e.g. Moscona and Garbor, '68) which condition the terminal expression of differentiating cells.

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