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Fibroblasts Against Cancer Cells *in Vitro*

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Abstract. In coculture experiments of HeLa cells with normal human fibroblasts, the parameters cell:cell ratioand feeding frequency can be monitored in a way that the growth of fibroblasts can flourish to the point where fibroblasts attack and destroy cancer cells.

The growth of malignant cells within the environment of normal cells in the organism indicates that the process guiding a cell to neoplasia eludes the physiological mechanisms which are responsible for the controlled growth and function of all interacting cell systems in the organism. These physiological mechanisms function efficiently during embryonic development, wound healing and in the adult organism, while in the cancer patient they are manifested in all cell systems except in the neoplastic focus. Cancer cells, therefore, acquire properties enabling them to overcome the normal state, disrupting the hierarchical relations within and among tissues, and to establish a new relationship with the normal cells which finally leads to the deterioration and destruction of the physiological state. Thus cancer is seen as a dynamic developmental disorder in which the organism falls behind in its ceaseless effort to maintain order (1,2).

It is still unclear how the organised state of a tissue reacts to the first signs of malignant development. When the tumour appears we usually admit that the organism has already lost the battle and no longer resists tumour growth. For metastasizing tumours this phenomenon obviously occurs not only in the primary tumour environment but also in other tissues susceptible to the establishment of metastatic foci, while in patients with non-metastasizing tumours the organism maintains the capacity to resist metastasis (3).

Hence, the physiological mechanisms for the prevention of malignancy should be sought rather in the normal tissues of healthy individuals or patients with evidently non-metastasizing tumours than in cancer patients with metastasizing tumours. Furthermore, at the cellular level, the close interactions between pairs or groups of different cell types in the organism seem to be a key area for such studies, since these interrelationships and cell-cell interactions are evidently disturbed in malignancy (4-8). An understanding of tumour growth control must rest upon knowledge of the growth and functional regulation of normal cells.

The fact that cancer cells come into continuous contact and inevitably interact with surrounding normal tissues in vivo implies that these normal tissues may have acquired a neutral or supportive attitude towards the cancer cells. With regard to this, there are indications that cancer cells modify their environment in favour of their further proliferation. Such modifications lead to the processes of tumour angiogenesis (9,10) and metastasis (11,12,13) and the induction of proliferation of connective tissue (14,15). The peculiar and still obscure growth control processes in neoplasia involve both the neoplastic and the normal counterparts of a tumour. The unpredictable variability concerning the speed of growth of a tumour, the

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development of its stroma, the histopathological pattern, and the appearance of invasive cells is a common experience. These variable characters constitute the heterogeneity of human tumours (16,17).

In breast cancer, as well as in most other tumours, the development of the stroma seems to play an important role. Stromal cells grow in close contact with cancer cells with a different speed in different tumours and the factors that control this growth have not yet been elucidated.

Three observations point to the significance of stromal fibroblasts isolated from human tumours: (a) their parallel growth with the neoplastic epithelial cells in the tumour (18,19); (b) their varying proportions in different tumours or in the same tumour type in different individuals (18,19) and (c) the presence of connective (fibrous) tissue in sites of completely regressed tumours (20,21). It has been suggested that stroma development represents a reaction of the immune system against the tumour and that it plays a role in impeding invasion (19,21,22).

Apart from these observations, since stromal fibroblasts are derived from mesenchyme, the elucidation of the influence of the mesenchyme on normal epithelial development and function (23) is of prime importance in order to answer the question "which common or uncommon features exist between the fibroblasts of normal tissues and tumours?" It has been shown that the histological differentiation and morphogenesis of an embryonic epithelium depend upon the influence of an underlying mesenchyme (23,24,25). Furthermore, epithelio-mesenchymal interaction is also implicated in the development of epithelial neoplasmata (26).

Thus stromal cells should be considered as an integral part of the problem of neoplasia. If we accept that stromal cells are developed as a host reaction to the neoplastic cells it would seem probable that an in vitro system for studying the behaviour and interactions of these two cell types might prove useful. A new direction was given to the study of the problem by the idea of replacing the stromal fibroblastic cells of a tumour with normal fibroblasts and studying their interaction with cancer cells in an in vitro model system.

Normal fibroblasts presumably differ from stromal fibroblasts at least in that they have never come into contact with neoplastic cells.

Thus their behaviour may parallel the behaviour of those fibroblasts in the organism that first come into contact with the first malignant cell and then expand to become the cell population called stromal fibroblasts. Increasing evidence suggests that fibroblasts derived from the skin or other body sites of cancer patients may in vitro exhibit different properties in comparison to fibroblasts of normal individuals. Such properties include their susceptibility to viral transformation (27,28,29) and their growth characteristics in vitro (29,30,31).

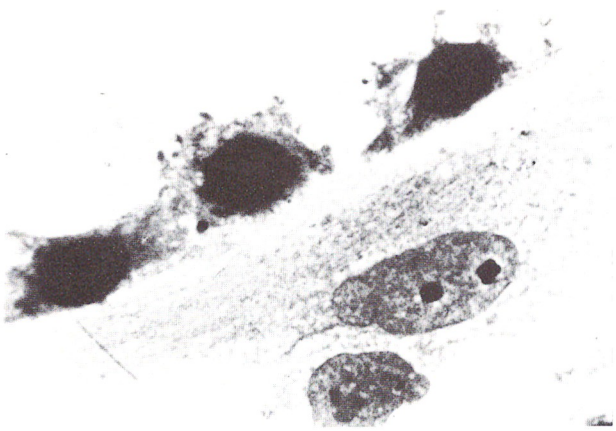
In previous experiments it was shown that fibroblastic cells derived from the stroma of human breast tumours present variable morphological and growth properties in vitro (31,32) which differ from normal skin fibroblasts derived from cancer patients or healthy individuals.

In a series of experiments on in vitro interactions between HeLa cells and fibroblasts isolated from human breast adenocarcinoma or fibroblasts isolated from human skin, the following observations were made (33,34):

(a) HeLa cells and fibroblasts act as chemoattractants for each other (Fig.1). Moving fibroblasts are preferentially directed towards HeLa single cells or colonies with the obvious tendency to encircle them (34). The chemotactic response of fibroblasts to HeLa (either single cells or colonies) may indicate the presence of specific recognition sites (receptors) on the cell membrane, their affinity to and availability for the chemoattractant (35,36). It has also been reported that tumour cells show enhanced chemotaxis to fibroblasts-conditioned medium (37,38), fibronectin (37) and collagen or collagen-derived fragments (39).

(b) HeLa and fibroblasts communicate exchanging ^{3}H-UdR and possibly other compounds or messages (34).

(c) When a dilute suspension of fibroblasts is added over a nearly confluent monolayer of HeLa, fibroblasts can find a place to attach themselves on the glass among the HeLa cells and HeLa cells respect these fibroblasts, not overlapping them (Fig.2);



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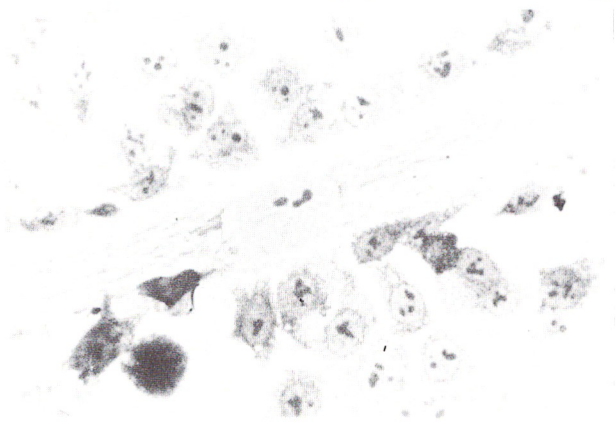

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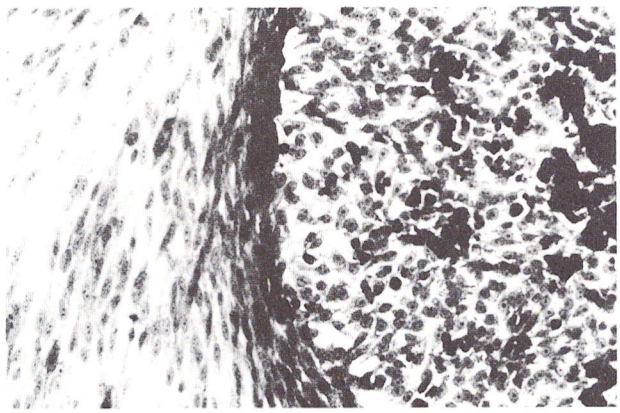

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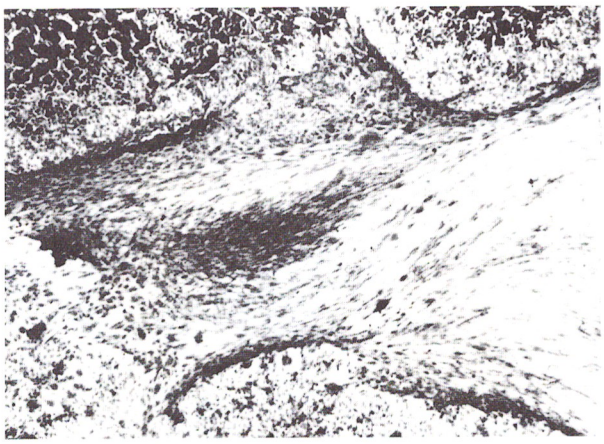

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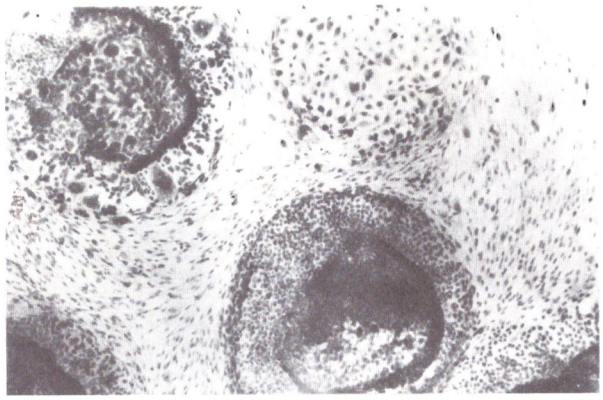

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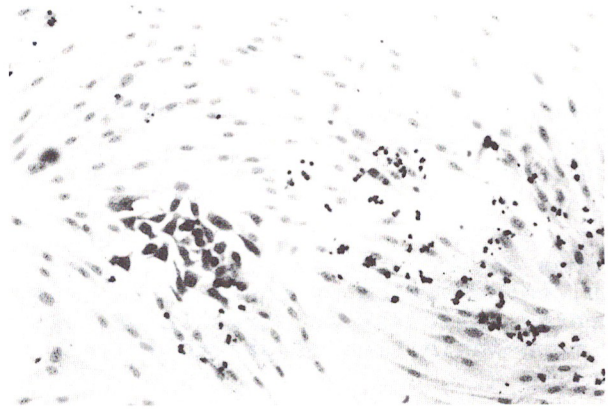

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Figure 1. Adherence of HeLa cells on the side edge of a fibroblast (G-EP) after 2 hours of co-culture. x 1500,Giemsa.

Figure 2. Absence of overlapping between fibroblasts (G-EP) and HeLa cells. Fibroblasts in suspension were added over a nearly confluent monolayer of HeLa and cultured for 48 hours. x 900, Giemsa.

Figure 3. HeLa cells and G-EP fibroblasts cultured at a ratio 1:10 (10^2cells per 8.03 cm^2) for 10 days. Overgrowth of both fibroblasts and HeLa at the edges of the HeLa colony give rise to the formation of a dense surrounding bow (DSB). x 240, Giemsa.

Figure 4. Fibroblast dense growth in fibroblast territories far from HeLa colonies (center) and formation of DSB around HeLa colonies. x 30, Giemsa.

Figure 5. Giant multinucleated cells in a HeLa colony (upper left) and two concentric DSBs in the HeLa colony (lower right). HeLa cells and fibroblasts were co-cultured (ratio 1:20,10^3 cells per 8.03 cm^2) for 14 days. Medium was not renewed for the last 6 days. x 25, Giemsa.

Figure 6. Disorganization of HeLa colonies, destruction of HeLa cells and formation of "mini cells" in a co-culture of HeLa VS.G-EP fibroblasts after 20 days. Moving fibroblasts invade the space previously occupied by HeLa. x 100, Giemsa.

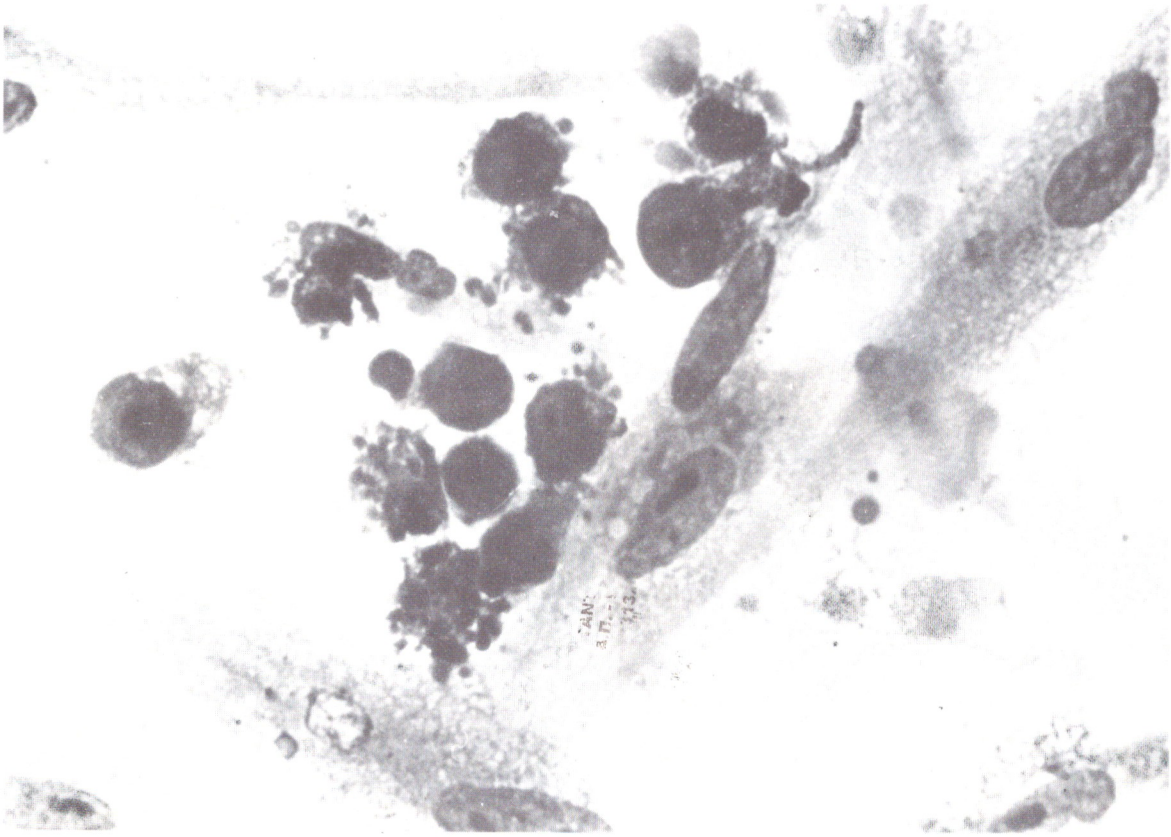

Figure 7. A focus of fragmented HeLa cells among fibroblasts with a normal appearance. HeLa and G-EP cells (ratio 1:10, 10^2 cells per 8.03 cm^2) were co-cultured for 12 days, with medium renewals every second day. x 1500, Giemsa.

connections develop between the two types of cells, but division of these fibroblasts is prevented obviously as a result of contact topo-inhibition of growth (34,40, 41).

(d) After 5-10 days in coculture, fibroblasts around HeLa colonies overgrow to form a dense surrounding bow (DSB) (Fig.3) (33).
Fibroblast dense growth with obvious overlapping is also seen in fibroblast territories far from HeLa colonies (Fig.4), phenomenon which never occurs in normal fibroblast cultures but does occur in stromal fibroblasts derived from breast tumours (31).

(e) At the edges of several HeLa colonies a high number of giant multinucleate cells is observed (Fig.5) (33).

(f) Fibroblast growth depends on space availability and medium replacement. If fresh medium is not provided, HeLa cells can invade the fibroblast DSB and form a new zone of HeLa cells edging out the surrounding fibroblasts (Fig.5). It is presumed that HeLa cells feed on fibroblasts since if old medium is replaced by new, fibroblast growth is again activated to form a second DSB (Fig.5).

(g) After the 12th day of coculture, many foci of destroyed HeLa cells are observed. Destruction of HeLa is evident from extensive nuclear and cytoplasmic fragmentation (Fig.7) (33). This phenomenon can not be reproduced by culturing HeLa in cell-free fibroblast-conditioned medium. Soon after the HeLa destruction the HeLa fragments round (mini cells) and show an affinity for the fibroblast cell surface (Fig.6). After the 20th day of

coculture, the HeLa colonies look highly disorganized and the space previously occupied by HeLa is now invaded by moving fibroblasts (Fig.6).

The phenomenon of the destruction of HeLa cells requires the following conditions:

(a) An appropriately low inoculum of cells, so as to allow cell-cell coculture for long periods (15-20 days) without subculturing in order to follow the morphological pattern of cell growth.

(b) A high ratio of fibroblasts: HeLa (10:1) in the mixed inoculum in order to avoid overgrowth of HeLa and prevention of fibroblast growth since fibroblasts have a longer replication cycle than HeLa.

(c) Medium replacement every 2nd day. This seems to be essential since when the medium is exhausted HeLa tend to feed on fibroblasts and, as discussed above, can invade the DSB.

These experiments show that when the parameters cell:cell ratio and feeding frequency, are monitored, the in vitro growth of fibroblasts can flourish to the point where fibroblasts attack and destroy cancer cells.

The questions arising now are:
(a) Does the same phenomenon occur in vivo as part of the host defence against cancer? and (b) Can in vivo conditions be modified so as to allow stromal fibroblasts or fibroblasts introduced into the tumour to attack the cancer cell territories?

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