Effects of Extracellular Matrix on the Malignant Phenotype

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Extracellular matrix molecules, including collagen, glycosaminoglycans (usually linked to a protein core as proteoglycan), elastin, and glycoproteins, influence the initiation and maintenance of differentiation of a variety of cell types. These molecules bind to the cell surface at specific sites and nonspecifically by electrostatic forces. Such interactions may alter the cell's response to growth and differentiation factors. After neoplastic transformation, most cells retain some dependence on these factors. This paper reviews the influence of matrix components on the phenotype of a variety of malignant cells and concludes that *in vitro* studies of malignant cell behavior require the utilization of an appropriate microenvironment.

The microenvironment of a cell is essential to the differentiation process. A major factor in the influence of the microenvironment is the continuing interaction of the cell surface with the extracellular matrix [1]. The basic structural composition of the matrix consists of at least four major classes of macromolecules, including collagen, glycosaminoglycans (usually linked to a protein core as proteoglycan), elastin, and glycoproteins, including laminin and fibronectin [1]. These molecules influence the initiation and maintenance of differentiation of a variety of cell types, including myoblasts [2,3], corneal endothelial cells [4], kidney epithelial cells [5], and hepatocytes [6]. Neural crest cells may express the mature phenotype or a melanocyte or a catecholamine-containing cell, depending on their environment [7].

It has been suggested that substrata can modify cell shape and orientation and allow cells to respond to naturally occurring hormones and growth factors to which they do not respond when maintained on other substrata [8]. For example, Gospodarowicz et al. [9] found that corneal epithelial cells adopt a flattened configuration when maintained *in vitro* on plastic and are very sensitive to fibroblast growth factor, but not to epidermal growth factor. When maintained on collagen, these cells become columnar with enhanced responsiveness to epidermal growth factor. Changes in cell shape have previously been associated with changes in DNA [10], RNA, and protein synthesis [11]. It has been suggested that the cell cytoskeleton may affect protein synthesis by dictating the arrangement of the polyribosomes associated with the microtrabeculae [12].

The cell-surface-extracellular matrix is believed to be a continuum without demarcation [13]. Matrix molecules bind to the cell at specific sites and nonspecifically by electrostatic forces [13]. In addition, matrix molecules have binding sites for other matrix components [14,15]. It has also been demonstrated that membrane-intercalated heparan sulfate interacts with polymerized actin [16].

Although the shape of transformed cells is not closely correlated with growth [10],

TABLE 1
Matrix-Induced Changes in Malignant Cell Phenotype

Cell	Substrata	Parameters	References
1003 embryonal carcinoma	Laminin	Growth, morphology, neurite extension, muscular differentiation	[18]
MKN-28 gastric carcinoma	Fixed fibroblasts	Morphology, glycosaminoglycans	[19]
Hepatoma	Collagen	Growth, morphology, tyrosine aminotransferase	[20]
B16 melanoma	Collagen I, IV	Morphology, glycosaminoglycans	[21]
	Laminin	Growth, pigmentation	[22]
MCF-7 mammary carcinoma	Collagen	Morphology	[23]
Acute myeloid leukemia	Marrow adherent cells	Growth, B4.3, myeloperoxidase	[24]
PC-12 pheochromocytoma	Collagen I or colon cancer matrix	Morphology, growth, neurite extension	[25]
HL-60 leukemia	Marrow matrix	Growth, morphology, esterase	[26]
U-343 glioma	Collagen I, IV or pia-arachnoid	Growth, morphology	[27]
BSp73-AS pancreatic cancer	Endothelial matrix	Morphology	[28]

neoplastic cells retain some dependence on the growth and differentiation factors of their environment. For example, the malignant behavior of embryonal carcinoma cells can be reversed under appropriate environmental conditions [17]. As shown in Table 1, a variety of malignant cells *in vitro* express phenotypic changes in response to substrata.

We have previously demonstrated the influence of the extracellular matrix on neoplastic cell behavior [21]. Thus, we have shown that B16 melanoma cells display altered morphology when grown on collagen I or IV substrata compared to cultures on plastic. On type IV collagen only, cells demonstrated an increased substrate adhesiveness. Incorporation of [^{3}H]-glucosamine and [^{35}S]-sulfate into glycosaminoglycans of cells grown on collagen substrata was 20 percent and 40 percent less, respectively, than cells grown on plastic. Although the composition of glycosaminoglycans was similar in all cultures, consisting of approximately 60 percent chondroitin and 40 percent heparin or heparan sulfate, the degree of sulfation of the heparin or heparan sulfate molecules was markedly decreased in cultures grown on collagen. Our results indicated that the composition of the extracellular matrix influenced the biological behavior of B16 melanoma cells, in part by altering the amount and nature of the glycosaminoglycan molecules produced. Similarly, endothelial cell-derived matrix has recently been demonstrated to alter proteoglycan surface antigen expression by human melanoma cells [29].

The role of the bone marrow microenvironment in the regulation of normal and malignant hematopoietic cell development is another example of cell-matrix interaction. The specificity of organ sites in which hematopoiesis occurs is demonstrated by the homing of transfused marrow cells to recipient mouse marrow and spleen [30]. Cells that go to the spleen form predominantly erythroid colonies and those in marrow form granulocyte colonies [31]. The need for a supportive environment was also demonstrated in the genetically anemic Sl/Sl^d mouse strain [32]. Their anemia is not

reversed by normal hematopoietic cells, but is cured by normal spleen [33] or marrow stroma [34].

In vitro studies also confirm the requirement for a supportive stroma [35]. Endothelial cells, fibroblasts, reticular-like cells, adipocytes, and macrophages from bone marrow form a layer which is adherent to the tissue culture flask and supports development of hematopoietic cells. In this environment, pluripotent stem cells can proliferate and differentiate to a variety of blood cell types, and such bone marrow cultures can be maintainted for several months. Non-marrow stroma is unable to support the hematopoietic process [36].

The stroma also appears to maintain control over neoplastic hematopoietic cells. *In vitro* studies demonstrated that freshly isolated myeloblasts express mature leukocyte surface antigens without morphologic maturation when grown in co-culture with adherent cells from normal marrow [24]. To investigate further the influence of bone marrow stroma on leukemic cell phenotype, HL-60 human promyelocytic leukemia cells were grown in the presence of extracellular matrix derived from normal human bone marrow stroma cells [26]. After six days in culture, cell number decreased by 54 percent in matrix-coated compared to uncoated flasks. Morphologic changes of the HL-60 cells on matrix included vacuolization, spreading of the cytoplasmic borders, and decreased ratio of nucleus to cytoplasm. These cells also developed nonspecific esterase activity and induced Fc rosette formation. Additionally, bone marrow matrix altered the response of HL-60 cells to differentiation inducers. For example, cells grown on matrix mature in the presence of 1 nM 12-O-tetradecanoylphorbol-13-acetate (TPA), a concentration that does not induce differentiation of cells in uncoated plastic flasks [37].

Since tumor cell-matrix interactions do influence cell phenotype and response to proliferative and differentiation factors, future *in vitro* studies of malignancy may require the utilization of adequate matrices for stabilization of phenotype and for more accurate prediction of *in vivo* therapeutic response.

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