

secondary cultures. Many secondary lines can be adapted to grow in suspension and are nonanchorage dependent.

Most differentiated mammalian cell lines (e.g., human fibroblasts such as WI-38 and MRC-5 that are licensed for human vaccine production) are *mortal*. These cell lines undergo a process called *senescence*. Essentially, the cells, for reasons that are not completely understood, will divide only for a limited number of generations (e.g., about 30 generations for the MRC-5 cells). Cells that can be propagated indefinitely are called *continuous, immortal*, or *transformed* cell lines. Cancer cells are naturally immortal. All cancerous cell lines are transformed, although it is not clear whether all transformed cell lines are cancerous.

Because of the linkage of cancer to cell transformation, the FDA had been reluctant to approve products made from transformed cells. However, transformed cells usually become attachment independent and can be propagated indefinitely in suspension culture, which is highly desirable for large-scale production in bioreactors. A little over a decade ago the FDA began to approve processes for production of products, such as the therapeutic protein, tissue plasminogen activator, from processes using immortalized cells. The approval of such processes has provided a major impetus for development of bioprocesses based on suspension cultures of animal cells.

Table 12.1 provides a summary of differences between nontransformed and transformed cells. One particular characteristic is *contact inhibition*. In two-dimensional culture on a surface nontransformed cells form only a monolayer, as cell division is inhibited when a cell's surface is in contact with other cells. Transformed cells do not "sense" the presence of other cells and keep on dividing to form multilayer structures.

Although mammalian cell lines have been the primary focus of work in animal cell culture, the two are not synonymous. Insect, fish, and crustacean cell cultures are evolving technologies. In particular, insect cell culture is unusually promising for biotechnological purposes. The baculovirus that infects insect cells is an ideal vector for genetic engineering, because it is nonpathogenic to humans and has a very strong promoter that encodes for a protein that is not essential for virus production in cell culture. The insertion of a gene under the control of this promoter can lead to high expression levels (40% of the total protein as the target protein). Most cell lines are derived from ovaries or embryonic tissue, although at least one differentiated cell line (a BTI-EAA blood cell line) has been maintained in indefinite culture for 25 years. Insect cell lines are not transformed but are naturally continuous. In contrast, senescence is observed with many fish cell lines.

**TABLE 12.1** Comparison of "Normal" and "Transformed" Cells

Normal	Transformed
1. Anchorage-dependent (except blood cells)	1. Nonanchorage-dependent (i.e., suspension culture possible)
2. Mortal; finite number of divisions	2. Immortal or continuous cell lines
3. Contact inhibition; monolayer culture	3. No contact inhibition; multilayer cultures
4. Dependent on external growth factor signals for proliferation	4. May not need an external source of growth factors
5. Greater retention of differentiated cellular function	5. Typically loss of differentiated cellular function
6. Display typical cell surface receptors	6. Cell surface receptor display may be altered