Establishment of melanoma cell lines from surgical specimens has been carried out in our laboratory for over 15 years. These cell lines have been used to study T cell-mediated response to autologous tumor and for investigating several aspects of the biology of human melanoma. Most of the lines have been characterized for expression of HLA class I and II antigens, adhesion molecules, integrins and melanoma-associated antigens. Some of these lines are being evaluated by RT-PCR for expression of genes coding for known tumor antigens recognized by cytotoxic T lymphocytes, such as the MAGE family (see Chapter 15) and differentiation antigens (Melan-A/Mart-1, Tyrosinase, TRP-1, gp100). Selected lines have been characterized for expression of genes coding for cytokines and growth factors. Some lines have been evaluated for susceptibility to anti-proliferative or antigen-modulating effects of various cytokines. Interaction with extracellular matrix and with endothelial cells has also been investigated, as well as metastatic activity in nude mice.

1. CULTURE CONDITIONS

All the cell lines established in our laboratory and described in this chapter were isolated from surgical specimens of patients admitted to our Institute. The current success rate in deriving stable cell lines from fresh tumor samples is approximately 35%. The number of live tumor cells is critical, and every effort should be made to minimize the processing time of the specimen. Well-differentiated, highly melanotic tumors are generally hard to establish in culture.
Melanoma cells from skin, lymph nodes or subcutaneous nodules are isolated by mechanical disaggregation as described (1). Using a scalpel, the specimen is cut into small fragments (approximately 3 x 3 mm) in a Petri dish containing RPMI 1640 medium supplemented with antibiotics. Each fragment is then gently squeezed in the barrel of a syringe. The resulting cell suspension is filtered through sterile gauze to remove large fragments, and then gently centrifuged. When there is abundant connective tissue, enzymatic digestion is preferred (1). The tissue fragments are resuspended in collagenase on a magnetic stirrer at room temperature for up to 2 h. Red blood cells are lysed with ammonium chloride. Separation of live cells is achieved by centrifuging up to 5 mL of the cell suspension layered on 2.5 mL of a Ficoll gradient.

Primary cultures are routinely set up in either 25 cm² flasks or 24 well plates in RPMI 1640 supplemented with 10% fetal calf serum (FCS). The presence of many adherent cells with spindle to dendritic morphology as early as 12 h after initial seeding is a good indication of success. Fibroblasts, often present as contaminants in primary cultures, can be removed by adding cholera toxin to the culture medium.

The primary culture is not split until the cells approach confluence, which can take between 7 and 30 days. Once the primary culture has reached confluence, the line is likely to become established as, in our experience, well proliferating primary cultures will consistently progress to a continuous cell line, Established cell lines are easy to maintain in culture. RPMI 1640 with 10% FCS is the medium of choice and a seeding concentration of 1–2 x 104/mL in 10 mL (for 25 cm² flasks) or in 30 mL (for 75 cm² flasks) is recommended. Cultures should be split before confluence, since most melanoma cell lines will show signs of degeneration soon after confluence is reached, and subsequently it is difficult for these cells to recover.

In our hands serum-free culture of melanoma cell lines is not easy, in contrast to the Wistar experience (see chapter 14). In most instances growth is markedly reduced or even totally abolished. Serum-free media can be used for a few days if necessary. Some cell lines can be adapted to continuous culture in serum-free medium, but these are exceptional.

2. PATHOLOGY

Table 1 describes 70 melanoma cell lines established in our laboratory. Most of the cell lines were isolated from lymph node metastases (48 lines), some from primary tumors (8 lines, indicated as “Skin”, in the specimen site column). Other sites include subcutaneous metastatic nodules (12 lines) and metastases in visceral organs (2 lines). Regional lymph nodes are the most frequent site of metastasis, accounting for the fact that 68 % of the cell lines