Culturing Pheochromocytoma Cells.

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Cell Biology of the Chromaffin Cell
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Pheochromocytoma cells from humans, rats and mice rapidly cease proliferating in primary culture. In addition, variable proportions of the tumor cell populations undergo spontaneous neuronal differentiation [1-3]. Establishment of pheochromocytoma cell lines is therefore a challenging task. Immunocytochemical staining for tyrosine hydroxylase (TH) and bromodeoxyuridine (BrdU) after BrdU pulse-labeling [4] provides a means for rapidly assessing the success of attempts to establish cell lines and avoids the pitfall of propagating irrelevant types of cells while performing biochemical studies on persistent pheochromocytoma cells that are progressively diluted with successive cell passages. In most instances, proliferation of TH-positive cells ceases within two weeks, although the cells persist in cultures maintained for many months (Figure 1).

The NGF-responsive PC12 cell line, established from a rat pheochromocytoma [5], has for almost 30 years served as a tool for many aspects of neurobiology involving normal and neoplastic conditions. Recently developed mouse pheochromocytoma (MPC) lines from neurofibromatosis knockout mice supplement PC12 cells and have generated additional applications [6]. Advantages of the mouse lines include expression of substantial levels of phenylethanolamine N-methyl-transferase and expression of high levels of the receptor tyrosine kinase, Ret, which is characteristic of sporadic and familial human pheochromocytomas but not of PC12 cells. Disadvantages include an apparently less stable phenotype. Some MPC lines respond to the Ret-activating ligand, glial cell line–derived neurotrophic factor (GDNF), by ceasing to proliferate and undergoing neuronal differentiation similarly to NGF-treated PC12 cells[7].

The phenotype of PC12 cells has been remarkably stable for cells maintained as originally described in detail [5]. However, diminished NGF responsiveness, decreased numbers of large secretory granules or loss of other desired traits has occurred in some laboratories, emphasising the importance of freezing and storing early passages of any cell line. The characteristics of the cells have also been affected by culture conditions, most notably a switch made in some laboratories early in the history of

the cell line from RPMI 1640 medium to Dulbecco's modified Eagle's medium (DMEM), which increases cell flattening and cell-substratum adhesion because of its higher Ca^{++} concentration. PC12 cells maintained in DMEM often regain characteristics of canonical PC12 cells when re-introduced to RPMI. The properties of the cells are also transiently affected by different lots of serum, particularly horse serum, which may inhibit NGF responses, and by high plating density, which inhibits NGF responses while increasing catecholamine content.

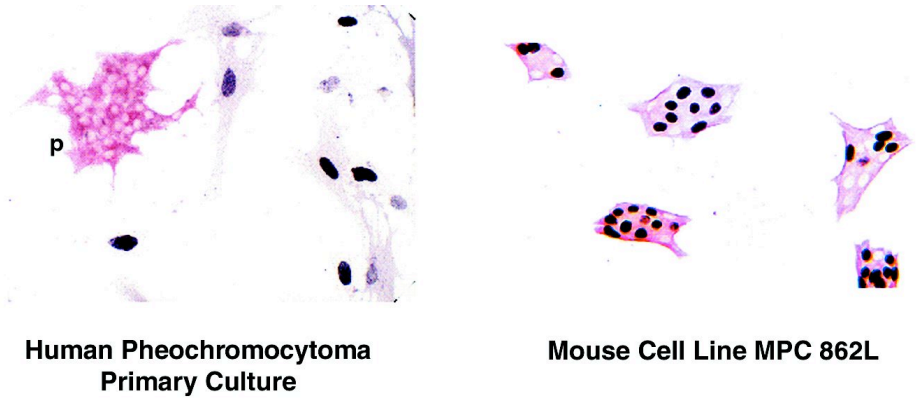

Figure 1. Double labeling for TH (pink cytoplasm) and BrdU (black nuclei) in primary culture of human pheochromocytoma compared to mouse pheochromocytoma cell line. A cluster of approximately 40 pheochromocytoma cells (p) in the human culture shows no BrdU incorporation, in contrast to adjacent, robustly proliferating but irrelevant cell types in the same culture and in contrast to the mouse cell line.

It should be borne in mind that PC12 cells and five of six lines of MPC cells arose from animals that had been irradiated postnatally, probably with resultant genetic damage that permitted the lines to be established. Pheochromocytoma cells from aged rats, non-irradiated *Nf1* knockout mice, MEN2B transgenic and *Rb* knockout mice as well as benign or malignant human pheochromocytomas persist in primary cultures but do not proliferate. This experience indicates that caution is warranted in drawing general conclusions from any single cell line, but

also suggests that understanding of factors that permit pheochromocytoma cells to proliferate might itself provide important insights for tumor biology.

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