Role of Intracellular pH in Proliferation, Transformation, and Apoptosis

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Both cellular proliferation and apoptosis (programmed cell death) have been claimed to be modulated, perhaps even triggered by, changes in intracellular pH. In this review, we summarize the evidence that gave rise to these hypotheses. To facilitate a critical appraisal of the existing data, we briefly review the main pathways involved in cytosolic pH homeostasis and their regulation by mitogens and by apoptosis-inducing agents. The information available at present suggests that cytosolic pH plays a permissive role in cellular growth and proliferation, but is neither a trigger nor an essential step in the mitogenic signal transduction cascade. Concerning apoptosis, it is clear that lowering the pH \textit{in vitro} can activate DNase II. However, the evidence linking cytosolic acidification with DNA degradation \textit{in vivo} is presently not convincing. We conclude that the cytosolic pH, an essential physiological parameter that is tightly controlled by multiple, complementary, or redundant systems, is unlikely to play a role in signalling either cell growth or death.

TUMOR pH AND THERAPY

Effective prevention and treatment of human cancer requires full understanding of the parameters that determine cell growth, proliferation, and death. One of the factors thought to control the rate of proliferation and the onset of programmed cell death is the cytosolic pH. In the following we will discuss the significance of the intracellular pH (pH\textsubscript{i}) and of the transport processes responsible for its homeostasis in relation to cellular proliferation, transformation, and apoptosis.

Comparatively crude, macroscopic measurements of pH have shown that the medium surrounding solid tumors is generally in the acidic range, though individual results can vary widely (see Griffiths, 1991 for review). The pH recorded is not only dependent on the type of tumor, but has also been found to vary spatially within the heterogeneous microenvironment of malignant tumors (Rockwell and Hughes, 1990). Extracellular accumulation of lactate is thought to be a major cause of tumor acidity. Regional hypoxia caused by variable blood flow and metabolism cause lactic acid generation in areas of tumors where anaerobic metabolism predominates. Furthermore, chemo- and radio-therapy influence the microenvironment of tumors and, conversely, regions with hypoxia and acid extracellular pH within a tumor influence the effectiveness of therapeutic regimes (Durand, 1991). Based on the unique microenvironment of solid tumors, treatment modalities designed to manipulate pH have been repeatedly suggested and applied clinically. Furthermore, rare instances of spontaneous regression of tumors have been speculatively ascribed to alterations of pH (Harguindey and Cragoe, 1992).

By extension of the findings made measuring macroscopic (largely extracellular) pH, the assumption was made that the cytosolic pH of tumor cells was also acidic. However, the advent of novel techniques for noninvasive measurement of intracellular pH has disproved this notion: the cytosolic pH of tumor cells...
is generally not acidic (Stubbs et al., 1995). Importantly, in the face of the well-documented acidic extracellular pH of tumors, tumor cells must be able to extrude protons more efficiently than their normal counterparts in order to maintain the cytosol near neutrality. In fact, the cytosolic pH of transformed cells has been often reported to be more alkaline than that of normal cells.

**pH-HOMEOSTASIS DURING CELL GROWTH AND TRANSFORMATION**

Cell transformation causing dysregulation of cell proliferation and/or cell death is a prerequisite for the development of a cancerous lesion. A causative link between cellular pH homeostasis and tumor development has been repeatedly suggested, and an elevated cytosolic pH has been demonstrated to parallel both cell transformation and proliferation (Doppler et al., 1987; Hagag et al., 1987; see also below). Therefore, it is important to understand the mechanisms whereby transformed or proliferating cells upregulate their capacity to extrude proton equivalents. Clearly, such knowledge could provide new targets for intervention in conditions where selective impairment of the growth of a subpopulation of cells is desirable, as in the case of cancer.

Cells have multiple mechanisms for pH regulation that are often regulated in concert (see Fig. 1). Such a redundancy of regulatory mechanisms most likely reflects the crucial importance of pH maintenance for overall cell function and survival. The following sections discuss the main pH homeostatic mechanisms that have been invoked in the control of cell proliferation and survival, with particular emphasis on the more recent literature. For an extensive review of earlier work pertaining to pH and cell growth the reader is referred to Grinstein et al. (1989).

**PROTON PUMPS**

All cells express vacuolar (V)-type proton pumps in endosomal, lysosomal, and Golgi compartments. In addition, a restricted number of cell types (e.g., urinary epithelial cells, osteoclasts, macrophages) express in their plasmalemma V-type proton pumps that contribute to cytosolic pH-regulation, transepithelial solute transport, and acidification of sealed-off extracellular spaces (Forgac, 1989). Proton pumping through the V-ATPase can be readily demonstrated by virtue of its specific and effective inhibition in the presence of the macrolide antibiotics bafilomycin and concanamycin.

The regulation of this multi-subunit proton transporter is poorly understood, but three separate lines of evidence suggest that dysregulation of proton pumping across the plasma membrane might be linked to a cancerous phenotype. First, transfection with a yeast plasmalemmal H^+-ATPase was shown to induce transformation of mammalian cells, possibly by elevating the cytosolic pH (Perona and Serrano, 1988; Gunn et al., 1994). Second, the relatively high cytosolic pH of some tumor cells has been correlated with and attributed to bafilomycin-sensitive proton transport across the surface membrane (Martinez-Zaguilan et al., 1993). Third, a transforming viral oncoprotein was shown to bind to a subunit of the V-ATPase both in vivo and in vitro (Goldstein et al., 1991). However, the following caveats should be considered: (i) The heterologous expression of a yeast proton pump in mammalian cells may have effects other than those directly related to alterations in the cytosolic pH. (ii) Bafilomycin-sensitive regulation of pH was demonstrated only in a minority of tumor cell lines tested. (iii) The functional consequences of oncoprotein interaction with the V-ATPase, in particular its effect on pH, have not been established. Thus, one must conclude that the evidence linking V-ATPase activity to