CARCINOGEN METABOLISM IN IMMORTALISED
HUMAN CELLS GROWN AS HYBRID CELLS IN CULTURE

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INTRODUCTION

Most cells from normal tissues have two characteristic properties. Their cell division is regulated in a particular way and they produce substances characteristic only of their tissue of origin. It was soon realised, however, that as cells from normal tissues were studied extensively, a limit to their long term cultivation was found. Normal cells died after a finite number of divisions. In contrast, tumour cells grew indefinitely in culture and usually did not express differentiated functions. In order to distinguish between these types of cells with finite or infinite lifespan in culture, Hayflick and Moorhead (1) used the term cell strain to denote normal cells with a finite lifespan and reserved the term cell line for cells which were established in culture and would divide indefinitely. They also noted that the property of infinite cell growth was usually associated with a change in the diploid nature of the cells and that a heteroploid karyotype was common in permanent cell lines.

More recently Pereira-Smith and Smith (2) have suggested that cellular immortality is a result of recessive alterations in the genetic program that limits the division of normal cells. Their studies further suggested that there was more than one way to alter this "normal program" in order that infinite life was obtained.

Our knowledge of the changes which take place when a cell with finite life in culture becomes immortal is far from complete but has been greatly increased by studies on cell transformation and cancer. Agents which cause normal cells to become cancer cells, e.g. chemicals and viruses, have been studied extensively and the various stages involved in the transformation of a cell are being defined. From studies on both DNA and RNA tumour viruses it is clear that an early event in cell transformation involves the establishment or immortalisation of the cell. For example, adenovirus is able to transform primary cells in culture in stages. The first stage is connected with immortalisation of the cells while the second is required for full expression of the oncogenic phenotype. The EIA proteins are the first viral polypeptides to be synthesised after infection and they are responsible for the immortalisation. This is thought to occur through their interaction with certain cellular proteins. One of the
most recent exciting discoveries in this field is the fact that the protein produced from the retinoblastoma susceptibility gene is one of the proteins the adenovirus protein EIA interacts with (7). Whatever the role of this retinoblastoma gene product, its loss by gene deletion or mutation or its effective removal by interaction with a viral protein produces the same end result—cell immortality. These studies are at an early stage and already other proteins as well as the 105KD retinoblastoma protein are being characterised as targets for the early adenovirus proteins (8,9). This work is now also being complemented by studies on SV40 and papilloma viruses and their transforming peptides. These, too, are able to bind to the cellular proteins identified in the adenovirus system (10,11,12).

The discovery of oncogenes in RNA tumour viruses has greatly increased our understanding of cell growth control and tumour initiation. Some oncogenes can encode proteins that are homologous to either growth factors or growth factor receptors, e.g. the oncogene v-sis (Simian sarcoma virus) is derived from the gene encoding one chain of the platelet derived growth factor (13,14). Also, the oncogene v-erbB (erythroblastosis virus) is derived from the normal epidermal growth factor gene (15). A further link between growth control and oncogene function is the common tyrosine kinase activity of many growth factor receptors and oncogene products (15,17,18). This relationship has motivated the research to explain non-virally induced cell transformation. Mutations which alter the expression of normal cellular genes, related to viral oncogenes, can have the same effects on cells as viral transformation. Thus, overexpression or mutation of particular cellular genes can lead to establishment or immortalisation of cells and cell transformation (19,20).

These studies on retroviral oncogenes have shown that the events involved in cell transformation are very complex. Initially it was suggested that the expression of a single oncogene, ras (rat sarcoma virus) was sufficient to immortalise and transform cells. Now it is apparent that oncogenes probably cooperate in the transformation process, i.e. expression of one oncogene may confer infinite life on a cell whereas a second oncogene product may be necessary for full transformation (20). This cooperation may involve DNA and RNA virus genes. Adenovirus EIA early gene products can immortalise cells which then become susceptible to transformation by the ras gene product (3).

Certain RNA viral oncogene products appear to mimic the action of the adenoviral early gene products. V-myc (myelocytosis virus) oncogene codes for a protein found in the nucleus of infected cells. The RNA transcript of its cellular counterpart, c-myc, has been reported to increase 10-20 fold in a variety of mammalian cell types in response to mitogenic substances such as growth factors and hormones (21,22,23).

In addition, decreased c-myc RNA levels have been reported in cells withdrawing from the cell cycle and undergoing terminal differentiation (24). Thus aberrant expression of the c-myc gene leading to overproduction of its RNA and protein product has been linked to uncontrolled cellular proliferation. The complexity of this problem, however, is exemplified by recent studies by Nath et al. (25) who have shown an increase in c-myc RNA in chick lens cells undergoing terminal differentiation and also in studies on hepatocytes from young and old rats it has been found that their responsiveness to epidermal growth factor is the same in that the c-myc gene is activated but the old hepatocytes did not initiate cell division. They traversed from G₀ to