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COMMENTARY

THE PHARMACOLOGICAL MEDIATION OF EPITHELIAL CELL PROLIFERATION

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Understanding the control of eukaryotic cell proliferation is one of the major challenges of modern cell biology. Successfully mediating the process of epithelial proliferation will represent a medical advance of widespread importance in controlling a number of diseases. First, the majority of human tumours are epithelial in origin. Second, a number of non-tumourigenic diseases result from abnormal control of epithelial growth. For example, in psoriasis, a common skin disorder, there is an increased rate of basal proliferation in the epidermis. The ability to mediate this proliferative process is also of major importance to elucidation of the mechanisms controlling regeneration and ageing and those controlling embryonic development and differentiation.

The cell cycle

To fully comprehend the problems involved in mediating proliferative processes, it is necessary to note the variability in cell turnover within the different tissues of the body. The process of nuclear division, or mitosis, was widely recognised long before the observation that DNA was synthesised during a discrete period, distinct from that in which mitosis was taking place. Hence, the concept of the cell cycle arose, in which the periods of DNA synthesis (S) and mitosis (M) are separated by two "gap" periods, G1 and G2 (for reviews see Refs. 1–3). Cells may continually cycle or enter a quiescent, non-proliferating state, designated G0, in which they remain viable and functional for long periods of time. Arrested cells from normal non-malignant tissue usually have the quantity of DNA found in G1 cells, and hence the G0 phase is often thought to be an extension of G1. When cells pass the G1-S boundary, they are usually committed to enter mitosis. The movement of the cells between the G1 phase and the quiescent G0 phase is an important regulatory phase of the cycle, and the extent to which this transition takes place varies greatly from tissue to tissue. For example, adult epithelial cells of the skin and gut undergo a continuous process of proliferation, migration, differentiation and death, whereas the adult epithelial cells of the liver and lung mainly exist in a resting or G0 phase. In these latter quiescent populations, the only normal entry into cell division is to replace cells lost during the slow process of cell turnover.

Experimental models for the study of cell cycle control

The difference in turnover rate of adult epithelial tissues has been exploited in the use of whole animal models of proliferation. The continually renewing systems of the gut and skin provide a means to assess the way in which normal proliferating populations recover from cell cycle perturbation by radiation [4], cytostatic drugs [5] and other mediators of cell proliferation [6]. These continually dividing sites are, inevitably, the epithelial sites most susceptible to damage during tumour therapy, since most antiproliferative agents are aimed at rapidly dividing cells. A further problem with this therapeutic approach is that a large proportion of tumour cells exist in a quiescent G0 state [7]. It has therefore been appropriate to study how normally quiescent epithelial populations may be stimulated into DNA synthesis. The mediators of this G0–G1 transition are often specific for a particular cell population. For example, proliferation of the epithelial acinar cells of rodent parotid and submandibular glands may be stimulated by the β-agonist isoproterenol [8]. Attention has been directed towards determining whether cells stimulated from quiescence and continually proliferating cells respond similarly to the cytocidal effects of anticancer drugs [9, 10]. Indeed, stimulating the cells of slow growing tumours into proliferation prior to therapy, with agents cytocidal to actively proliferating cells, has been used to enhance the effectiveness of drug treatment [11].

The regenerating liver

In certain tissues epithelial cells may also be surgically stimulated to undergo a G0–G1 transition. Hepatocytes have an exceptional capacity for this kind of response which, although termed regeneration, is more correctly compensatory hyperplasia, because the excised lobes do not regrow, the restoration occurring in the remaining lobes. Regrowth of the rat liver after excision of the median and left lateral lobes has been studied extensively as a model for epithelial proliferation. The time course of this response has been well-characterised in the rat [12]. DNA synthesis begins after a pre-replicative period of 16 hr and reaches a peak at 24 hr after partial hepatectomy. Mitosis begins at 24 hr after surgery and peaks at 33 hr. The original tissue mass is restored within approximately 1 week depending upon the age of the rat [13].

This model provides a unique system for probing the mechanisms controlling epithelial proliferation. Both factors which enable initiation of the proliferative response and those which enable proliferation to cease once the original mass of the liver

has been restored may be studied. This situation is clearly different from tumour cells which acquire an indefinite capacity for proliferation. If explants of liver are grafted to other parts of the body, they, too, are capable of responding to partial hepatectomy [14], providing evidence that circulatory factors are involved in the response. Indeed, a number of hormones, in particular insulin [15], have been shown to mediate liver regeneration. There is also evidence that suggests a regulatory role for nerve-derived factors [16], and it has been demonstrated that liver cell proliferation occurs in response to cycloplasmic reduction of hepatocytes as well as to actual cellular loss [17]. This points to the possibility of a liver cytoplasmic-derived inhibitor of hepatic regeneration.

Tissue culture models

The mechanisms involved in a proliferative response such as liver regeneration are extremely complex and highlight the problems of studying the effects of exogenous agents in whole animal models of proliferation. It is often impossible to assess whether an agent acts directly on a particular tissue or whether the response is secondary to regulatory effects elsewhere in the body.

Identification of pharmacological mediators of proliferation and elucidation of their mechanisms of action may be greatly simplified by the use of tissue culture models. Each type of system available has its own advantages and disadvantages. Primary cultures of dissociated cells remain closer to their *in vivo* counterparts with respect to growth properties. Cultures from heterogeneous tissues may be enriched for a particular cell type, providing opportunity for more defined experimental conditions than in the whole animal experiments. Cell lines, by definition, have lost important features of normal proliferative control, but it is often appropriate to compare growth mechanisms in non-tumourigenic cell lines with those of a tumourigenic line from the same tissue. Many experimental systems using cell lines involve growth arrest of normally proliferating cells by serum starvation or by density-dependent inhibition. Mechanisms enabling release from this arrested growth state are then studied. How closely this approach mimics entry of normal cells into the proliferative cycle is not known. The major advantage of using cell lines as an experimental model is that a cell system may be chosen which consistently or uniquely responds to a particular test factor. For example, the A431 cell line, whose expression of the epidermal growth factor (EGF) receptor is greatly amplified, has been used extensively as a model to study the mechanisms of action of EGF and the interactions of this growth factor with its receptor.

This commentary has so far been concerned with the experimental systems available to study the control of epithelial proliferation. This emphasis has been adopted because much of the progress in our understanding of this process may be attributed to the establishment of defined systems in which the serum factors affecting survival, differentiation and growth of cells can be identified [18]. A wide variety of physiological factors are capable of mediating epithelial cell proliferation in an interactive manner. Most of these factors may be effective pharmacological mediators of proliferation when applied to the appropriate cell type.

Hormones and growth factors

The ability of serum to maintain growth of cells in culture may be attributed to the presence of hormones and growth factors which regulate proliferation in a dose-dependent manner. For epithelial cells the most potent regulators are often insulin and EGF. The growth promoting activities of insulin are mediated by interactions with the receptors to the somatomedins (also termed insulin-like growth factors, IGF1 and IGF11) [19]. The physiological source of EGF is still unknown and, in this respect, all the data available concerning control by this factor may be considered as "pharmacological". The majority of epithelial tissues, including those of the lung and kidney, hepatocytes, keratinocytes, corneal epithelium and mammary gland epithelium, respond to EGF. A number of transformed cell lines also produce transforming growth factors (TGFs) which compete with EGF for its own receptor.

Platelet derived growth factors (PDGFs) are more widely mitogenic to connective tissue cells than to cells of epithelial origin. However, there are examples of platelet-associated growth activity in epithelial systems. A platelet-derived hepatocyte growth promoting factor has been identified in rat serum [20]. This factor is both biologically and physically distinct from human PDGF which is not mitogenic to rat hepatocytes.

The hormone arginine vasopressin (AVP) has been shown to exert proliferative effects on cultured 3T3 fibroblasts [21] although specific vasopressin receptors have not yet been demonstrated on these cells. In contrast, AVP receptors have been identified on epithelial cells, in particular those of the kidney and liver. AVP has been shown to regulate hepatocyte entry into DNA synthesis, both in primary culture [22] and *in vivo*, the rate of DNA synthesis following partial hepatectomy being reduced in the Brattleboro rat, which lacks hypothalamic AVP, compared with the rate in control Long Evans rats [23].

In a number of endocrine glands, there appears to be a correlation between secretion by the glandular epithelial cells and their rate of proliferation. A decrease in secretion is often accompanied by a decreased rate of proliferation and an increase in secretion accompanied by increased proliferation [24]. Responses of this kind have been noted in the pituitary, thyroid, adrenal cortex and gonads. However, despite the correlation between secretion and gland size, adrenocorticotrophic hormone (ACTH) is unable to induce compensatory hyperplasia after unilateral adrenalectomy [25] and has been shown to be growth inhibitory *in vitro* [26]. These observations led to the discovery that pro-opiocortin, the N-terminal precursor of ACTH, is capable of stimulating adrenal growth [27]. It may be that, in other endocrine cells, specific factors distinct from the secreted hormones are capable of regulating the proliferative activity that accompanies secretion.

Growth hormone release inhibitory hormone

(somatostatin) inhibits endocrine secretion at pharmacological doses. Long-acting analogues of this peptide hormone are clinically effective in reducing the secretions of pancreatic endocrine tumours [28] and vasoactive inhibitory peptide (VIP)-secreting tumours [29]. In the latter study, actual tumour regression was reported in response to this treatment. It is possible that the hormones secreted by these tumours exert autocrine growth stimulatory effects, enabling the cells to promote their own survival. If this is the case, then it follows that inhibition of secretion would also inhibit tumour growth. Further evidence for this kind of autocrine control has been found recently in human small cell lung cancers [30]. These tumours are able to secrete a variety of peptides including bombesin-like factors. Bombesin-like peptides were found to stimulate proliferation in cell lines derived from small cell lung cancers. Furthermore, an anti-bombesin monoclonal antibody, which was able to compete with this peptide for receptor binding sites, was also able to inhibit the proliferative response.

Nerve-derived factors

Peripheral nerves have been shown to exert long term trophic actions on some tissues [31]. Nervous control of proliferation is also implicated in epithelial tissues, for example, as previously mentioned, during the process of liver regeneration. Much of the data available has been obtained from neuronal lesioning experiments and has been interpreted by considering the roles of the classical peripheral neurotransmitter, acetylcholine, in the parasympathetic system and catecholamines in the sympathetic system. However, a number of other substances have now been proposed as peripheral neurotransmitters or neuromodulators, in particular, the neuropeptides.

Several peptides, including somatostatin, VIP and bombesin, were discussed in the last section of this commentary, with respect to their hormonal and autocrine roles in epithelial proliferation. It is important to note that these hormones are part of a large family of peptides which has also been demonstrated within peripheral nerves by immunochemical methods. It has been suggested that circulating somatostatin and locally secreted VIP and bombesin are able to exert trophic actions. This opens the possibility that similar peptides of a nerve tissue origin may also play a role in regulation of the cell cycle.

Many immunoreactive neuropeptides have now been demonstrated to coexist in the same cells as classical neurotransmitters. For example, somatostatin-like immunoreactivity has been demonstrated in noradrenaline containing cells in prevertebral sympathetic ganglia [32]. More recently [33], a vasopressin-like peptide (VLP) has been demonstrated within sympathetic ganglia and peripheral nerve fibres, including those of the liver and kidney. It may be that the trophic actions elicited by AVP may also be elicited by this larger nerve-derived peptide.

Mechanisms of proliferative control

It is clear that a very large number of purified substances are capable of stimulating epithelial proliferation under appropriate circumstances. It is also clear that many of these effects can be interactive and synergistic. For example, in cultures of rat hepatocytes, entry into DNA synthesis may be stimulated with various sera, EGF or insulin [34]. A number of other agents, which are unable to produce a response alone, are able to potentiate the response to EGF and insulin. It will therefore be necessary not only to understand the mechanisms responsible for initiation of a proliferative response but also to understand the ways in which the interaction of other agents with their own membrane receptors can modulate this primary response.

Attention has been paid to the possibility that changes in intracellular pH may be a common denominator in the mechanisms of mitogenic actions. This is based on rapid activation of the sodium/proton exchange pump following exposure of 3T3 fibroblasts to mitogens [35]. Another mechanism proposed for mitogenic growth factors was stimulation of tyrosine kinase activity, particularly with respect to EGF and PDGF [36]. The difficulty with these mechanisms is that evidence is largely correlative, showing rapid effects occurring before the stimulation of entry into DNA synthesis. It will be interesting to establish whether both sodium/proton exchange and tyrosine kinase activation are really necessary in the cascade of events in a mitogenic response, or whether they are simply the by-product of a more proximate stimulatory event.

As the best established secondary messenger system, the role of cyclic nucleotides in cell proliferation has been studied extensively. In some epithelial systems, for example the MDCK kidney cell line [37], the cAMP system may be a primary modulator of cell proliferation whereas in other systems, for example cultured epidermal keratinocytes [38] and mammary epithelia, the presence of serum is required before proliferation may be modulated by cyclic nucleotides.

More recently, the class of receptors linked to intracellular calcium mobilisation has been proposed to activate a secondary messenger system, by the regulation of the breakdown of plasma membrane inositol phospholipids [39, 40]. This pathway results in a number of different signals which are implicated in the control of proliferation. Receptor stimulation of this pathway results in production of diacylglycerol and inositol tris phosphate (IP3). Diacylglycerol has been shown to be a potent regulator of the enzyme protein kinase C [41]. This is of interest because the phorbol ester tumour promotors, which regulate differentiation and proliferation in a number of systems, are direct activators of protein kinase C [41]. Indeed, protein kinase C has been demonstrated to be a receptor for radiolabelled phorbol esters [42]. Therefore, receptor stimulants which regulate inositol phospholipid breakdown also regulate protein kinase C, which is known to exert proliferative control.

The role of calcium as a signal in the onset of proliferation has been recognised for some time [43, 44]. It is now known [45–47] that the other product of inositol phospholipid breakdown, IP3, can act as a signal to release intracellular calcium from the endoplasmic reticulum. Hence, this pathway may be important in regulating the calcium

signals involved in eliciting a proliferative response. Several agents, including dibutyryl cyclic AMP and forskolin are able to directly activate the cyclic nucleotide pathway. The development of similar agents for pharmacological activation of IP3 will enable direct evaluation of the importance of this pathway in proliferative control.

Summary

In the past, it has been necessary for pharmacological intervention of epithelial proliferation to mostly be limited to non-specifically arresting or killing actively proliferating cells. As our understanding of the mechanisms involved in mediating the processes of growth and differentiation increases, we can hope to see the development of a new pharmacology, in which proliferation of individual systems may be regulated in a less drastic manner.

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