EFFECTS OF RETINOIDS ON GROWTH AND DIFFERENTIATION

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1. INTRODUCTION

The retinoids constitute a group of compounds which includes vitamin A (retinol), its metabolites and a multitude of synthetic analogues. Retinol can be oxidized to retinal and these two retinoids are essential for vision and fertility. Apart from eliciting these activities upon cells of the visual and reproductive systems, retinoids appear to have a much more general influence upon cellular behavior: they affect the propensity to differentiate and proliferate. Much of the information to support this view is derived from studies with cultured cells (reviewed in 1). However, it was recognized long ago (2,3) that in animals on diets lacking retinoids, epithelial cells showed evidence of both metaplasia and hyperplasia.

In culture, retinoids tend to promote differentiation, and suppress proliferation, of cells (1), although there are exceptions (reviewed in 4 and 5). In general, retinoic acid is a considerably more potent modulator of gross cellular behavior than is retinol; on the other hand, circulating levels of retinol are two or more logs higher than that of retinoic acid. This raises several questions about the mechanisms by which retinoids influence cellular phenotype in vitro and in vivo. Some of these issues are addressed below, in most instances with mouse embryonal carcinoma cells, the stem cells of teratocarcinomas, as a source of information. We have concentrated our studies on these cells because they resemble cells of the early embryo in several respects, including the ability to differentiate broadly, because they differentiate readily in response to retinoids (as well as several other chemical inducers), and because they are rapidly proliferating, malignant cells whose differentiated progeny tend in most instances to be slower-growing and benign, making them a useful model system for testing the concept of differentiation therapy of cancer.

2. HOW DOES RETINOIC ACID PROMOTE DIFFERENTIATION?

All-trans-retinoic acid can induce differentiation in vitro of cells such as embryonal carcinoma cells (6,7) and promyelocytic leukemia cells (8) at concentrations in the nanomolar range. Our laboratory has presented several lines of evidence to implicate the cellular binding protein for retinoic acid (CRABP) in the induction of differentiation: (a) all of several tumor-derived embryonal carcinoma cell lines tested possess CRABP (9); (b) various studies have established a good correlation between the ability of acidic retinoids to bind CRABP and to promote differentiation of embryonal carcinoma cells (e.g., 10,11); (c) mutant embryonal carcinoma cells lacking or possessing much reduced levels of CRABP have little or no ability to differentiate in response to retinoic acid (12-14); and (d) differentiation response to retinoic acid can be restored in mutant embryonal carcinoma cells that regain CRABP activity by cell fusion (15) or treatment with sodium...
butyrate (16). cDNA for bovine CRABP gene has recently been identified and placed in a eukaryotic expression vector (17,18). In collaboration with Drs. Lena Wei and Chi Nguyen-Huu we are attempting to restore the ability of CRABP- embryonal carcinoma cell mutants to differentiate in response to retinoic acid by transfection of the cells with the expression vector.

How might CRABP mediate retinoic acid induction of differentiation? It appears likely that CRABP is an intracellular transport protein for retinoic acid. Following fractionation of cells exposed to retinoic acid, the retinoid can be found associated with most organelles (e.g., 19). However, control studies suggest that a similar distribution can occur when cells are exposed to [3H]retinoic acid at 4°C immediately prior to disruption (20). Thus, it is necessary to distinguish between an artifactual organellar distribution of retinoic acid due to its lipophilicity and true organellar interaction. In this regard, studies have demonstrated that retinoic acid can associate in an unsaturable, non-specific manner with isolated nuclei but apparently bind specifically to a finite number of nuclear sites when complexed with CRABP (21-24). It is conceivable, therefore, that CRABP transports retinoic acid into nuclei to interact with, and activate, genes which modulate cellular behavior, including genes which initiate differentiation of embryonal carcinoma cells. Such an activity is unlikely to be strictly analogous to the interaction of steroid receptor holoproteins with the genes they activate (e.g., references 25,26) since the CRABP appears to dissociate from the nucleus once it delivers retinoic acid to its specific sites (23).

No genes have yet been identified as being directly activated by retinoic acid. The observation that retinoic acid induces its own metabolism in cells which possess CRABP but not in CRABP- embryonal carcinoma cells would be consistent with the view that the CRABP holoprotein activates the gene(s) (presumably in the cytochrome P450 family) responsible for the metabolism (27,28). We have obtained preliminary evidence that the mutant cells become competent for induction of retinoic acid metabolism when they regain CRABP activity (29). However, our studies also show that some mutant embryonal carcinoma cells possess CRABP and the ability to induce retinoic acid-metabolizing enzymes but still fail to differentiate upon exposure to retinoic acid (28).

3. HOW DOES RETINOL PROMOTE DIFFERENTIATION?

In initial studies on retinoid induction of differentiation of embryonal carcinoma cells it was reported that retinol was without effect (6). In subsequent investigations, however, it was demonstrated clearly that retinol could promote differentiation of embryonal carcinoma cells, albeit considerably less efficiently than retinoic acid. (30-32). In view of the above discussion of retinoic acid, it is possible that retinol promotes differentiation (a) via metabolic conversion to retinoic acid, (b) by an analogous mechanism, or (c) by unrelated means. There is evidence to suggest that retinol action depends upon metabolism to retinoic acid. Williams and Napoli (32) reported that retinol was about 0.5% as potent as retinoic acid in inducing differentiation of F9 embryonal carcinoma cells using laminin production as an indicator of differentiation; these authors claimed that this potency correlated well with the percent conversion of retinol to retinoic acid by F9 cells. A consistent finding is that embryonal carcinoma cell mutants that had lost responsiveness to retinoic acid also failed to differentiate when exposed to retinol (19).

Other data do not support the view that retinol promotes differentiation of embryonal carcinoma cells via conversion to retinoic acid. Gubler and