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REVIEW ARTICLE

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Retinoic acid, neoplasia, differentiation and development

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Abstract Retinoic acid has pronounced effects on cultures of neoplastic cells. These have attracted the attention of pathologists, but it is important to note that much of the critical data about retinoic acid has been obtained from the current extension of our knowledge in the field of development. Some of these changes are reviewed here.

Key words Retinoic acid · Development · Differentiation · Neoplasia

Introduction

In a recent paper in the journal Kloppel and co-workers [4] have examined the role of retinoic acid (RA) as a modulator of differentiation in various cell lines in pancreatic carcinoma. RA has been used to induce growth suppression in Wilms' tumour [23] and the capacity of retinoids to induce phenotypic change in a number of epithelial and haematogenous malignant states is well established. However, much of our knowledge about the action of retinoids in maintaining the differentiated state has come from those working in developmental biology. The retinoids are major morphogens and it is worth reviewing data from developmental studies to see how pathology is informed by experimental embryology. As a specific endpoint which is an example of this interaction, in a fascinating study illustrating the links between development and neoplasia, Perez-Castro et al. [14] have found that overexpression of the cellular retinoic acid binding protein 1 (CRABP I) gene in the mouse results in pancreatic tumours of the islets of Langerhans.

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Morphogens

It is generally accepted that the development of multicellular organisms depends on an exchange of signals between cells that result in local changes in gene expression, rather than the slavish following of a DNA dependent programme analogous to constructing a factory from a blueprint [2]. Chemotactic factors are often invoked in accounts of this aspect of development and positional information for cells is thought to be provided by concentrations of chemical morphogens that act across small fields (circa 30–50 cells per field). In the last 10 years these compounds have begun to be identified, but to allow this process to occur it has been necessary to define a morphogen more carefully than in some older classical embryological texts. Lumsden [12], writing about the CNS, suggested the following criteria for morphogen identification: the factor should be regionally specific, must be available at the appropriate time in development and be produced at a level sufficient for free diffusion and the establishment of a gradient over distances that (nerve fibres in his example) grow to meet their targets. It is clear that these criteria can be met by the retinoids (see, for example, Wagner et al. [24]).

The retinoids and their receptors

The term "vitamin A" is often used to refer to retinoid compounds that have the biological activity of retinol. These compounds are mainly found in dairy products and liver but β-carotene and the carotinoids are precursors of retinol occurring in vegetables, party converted to retinol after ingestion. Other sources include food supplements, which may affect intake significantly – the daily requirement of around 800 retinol equivalents (2700 IU of vitamin A per day) may easily be exceeded by those eating conventional diets. RA is a minor component of the diet and both all-*trans*-RA and 13-*cis*-RA are metabolites of preformed vitamin A.

There are two families of nuclear retinoid receptors, RAR and RXR. These are all-*trans* and all-*cis* (RARs) or 9-*cis* (RXRs) RA dependent transcription factors which act by binding to specific DNA sequences (RA response elements). At least three genes have been identified for each family and each gives rise to multiple isoforms via alternative splicing, some of which are inducible by RA. The formation of different heterodimers gives rise to a wide variety of isoform combinations which probably have specific regulatory functions.

The gene family encoding the receptor proteins is in two main functional groups: one for the steroids oestrogen, glucocorticoid, progesterone, aldosterone and vitamin D; the other consists of 6–7 genes, two of which encode thyroid receptors [16]. All members of the family are related to the viral oncogene *erb*-A1 and one of the thyroid receptors (TR-α) is encoded by the cellular *erb*$_{A1}$ gene. It is to this group that the RA receptor belongs. Petkovich et al. [15] and Giguere et al. [7] first identified the cytosolic RA receptor as a member of the steroid/thyroid hormone receptor family.

In addition to the nuclear receptors for RA there are two highly conserved cytosolic RA binding proteins: CRABP I and CRABP II. They are similar, but CRABP II binds RA less effectively. In an interesting paper Ruberte and her co-workers [20] have shown that the CRABP I protein is found at sites sensitive to RA-induced teratogenesis (neural crest and hindbrain) and suggest that these cells cannot tolerate high levels of RA in their normal development. CRABP II was found in these sites but also in sites not known to be especially vulnerable. The genes for RA receptors and the CRABP II protein are co-expressed in tissues which lack CRABP I. This latter protein appears to be involved in the control of levels of free RA available to nuclear receptors and the two C proteins and the receptor expression are all influenced by RA itself. The Chambon group [9] have shown that null mutant mice deficient in one or both forms of CRABP are phenotypically normal and they suggest that the role of the CRABPs is to maintain physiological levels of intracellular RA under conditions of limited supply of RA. However, Fawcett et al. [5] have found that, depending on the genetic background of the animal, postaxial polydactyly (often unilateral) is consistently induced by loss of the CABP II gene.

A summary of the mode of action of this system might be made as follows. Two classes of cellular proteins bind all-*trans* RA, the nuclear RA receptors and the cellular RA binding proteins (CRABP I and II). The RA receptors regulate gene transcription by binding to DNA. The cellular RA-binding proteins act by regulating the amount of free RA reaching the nucleus of a given cell (preventing binding with the nuclear retinoid receptors).

Development and the retinoids

In Man, a well-defined RA embryopathy is characterised by abnormalities in cranial neural crest derived structures (microtia, anotia, migrognathia); cardiac anomalies (cono-truncal and arch) and thymic abnormalities (aplasia, ectopia) are also common. There is also evidence for non-crestal CNS cellular changes, with cellular ectopia common in the cerebellum. Very similar patterns are seen in animal models, which is an uncommon finding; teratogenesis is not a process in which trans-species phenocopies are common. This fact perhaps identifies the involvement of an essential and important developmental mechanism.

Isotretinoin (Accutane;13-*cis*-RA, a treatment for severe acne) is the preparation most commonly associated with anomalies in Man [18] but concerns for the population have been directed towards dietary ingestion. In a large study Rothman et al. [19] identified 22 748 women as they underwent antenatal screening and obtained histories of their diet, medication, illnesses, family history and exposure to environmental agents. Three hundred and thirty nine babies had birth defects – 121 of a type indicating involvement of the neural crest. There appeared to be a threshold for teratogeneic effects at around 10 000 IU/day of supplemental vitamin A in this group of 121 babies and there was evidence of effect mainly in those infants exposed in the first 7 weeks of gestation. The authors estimate that 1 in 57 infants born to women who took more than 10 000 IU of preformed vitamin A/day in the form of supplements were affected.

The development of the limbs

This toxicological accident focused clinical attention on the role of retinoids in neural crest development but the importance of RA had first become obvious in the studies of development of the limb.

Two different mechanisms define the antero-posterior and proximo-distal positional values in the limb. The antero-posterior values are determined by a morphogen produced by the cells of the polarising zone (PZ) at the distal posterior margin of the limb bud [3]. Cells which lie close to this zone receive a high dose of morphogen (now known to be RA) and form structures which are posterior in type – such as little fingers. At the opposite end of the gradient they will form thumbs. Grafting experiments show that this pattern can be manipulated readily by the transposition of groups of cells from the PZ or by implantation of beads loaded with RA; receptors for this morphogen are found in the limb bud at appropriate sites and stages. Thaller and Eichele [21] first reported that gradients of RA specify the antero-posterior digit pattern in the chick wing and subsequently [22] that a number of retinoids may be effective in this system. Local application of all-*trans*-RA to the anterior margin of the chick limb bud results in the formation of structures like those produced if cells from the PZ are transplanted to the same site. Theoretically the RA may work directly on limb bud cells by giving them positional information or it might create a PZ and in an elegant experiment Helms et al. [8] showed that the latter expla-

nation was the correct one, using beads soaked in RA and also demonstrating a dose-dependent effect, but at non-physiological levels.

Proximo-distal positional information depends on a different system – the actions of the apical ectodermal ridge (AER) at the distal end of the limb bud. This maintains the mesoderm deep to it in a state where the cells display an autonomous tendency to become progressively more distal in their positional behaviour with time. As the limb grows, groups of cells become displaced from this specialised mesoderm and their positional value is thus fixed.

The way in which these systems interact is not entirely clear. It seems probable that RA may regulate homeotic gene expression and that this compound may be able to reset positional values experimentally [10]. Cells in the posterior part of the limb bud that will give rise to posterior structures such as little fingers are found to express all of the homeobox genes in the Hox-4 complex in the chick, while cells from which anterior structures develop express only Hox4.4. FGF-4 is necessary to maintain the polarizing activity of the cells in the PZ; this is apparently produced by the AER, providing a link between these two important organising regions.

Non-limb-related effects

The first observable time of action of retinoids as a major morphogenic influence is far earlier than one might suppose intuitively when thinking about their role in the formation of the limbs. The elegant work of Marshall et al. [13] using transgenic mice in which a reporter gene was linked to the promoter for one of the Hox genes involved in the development of the brain (Hox 2.9) has shown that RA produces anterior expansion of the area where the gene is expressed with repetition of a sequence of rhombomeres replacing anterior segments. Here we have a gene that clearly specifies a region of the brain and related structures in the head (the respecification is accompanied by respecification of associated neural crest derived structures) which is affected by RA treatment in the gastrula stage. Gale et al. [6] have shown that later exposure of the developing brain to RA alters the behaviour of the two cell populations in the rhombomeres (neural crest cells and neuroepithelium) selectively; the pathways of migration of the cell processes of certain sensory ganglia are affected but motor neurons and mesenchymal crest migrations are not.

However, the retinoid system is also involved later in CNS development. Rodriguez-Tebar and Rohrer [17] have shown that the phase of non-dependence of peripheral and sensory neurons to nerve growth factor (NGF) is ended by the induction of high-affinity receptors by RA. This dependence is critical to subsequent morphogenesis in the nervous system; at a time when outgrowing neurites meet their targets, they will respond to signals produced by target-cell-derived NGF and this will play a part in the adjustment of the innervation density of the target.

Thus at the gross and fine end of the developmental framework, retinoids have roles. These roles are ancient; it seems that the retinoids and their receptors assumed a central role in development long ago. The group of Chambon [11] have found embryonic and fetal death together with ocular, CNS, skeletal and previously non-retinoid associated malformations in RAR compound null mutants. Defects of the axial skeleton in these animals are of particular interest in that ancestral features typical of reptiles are found, suggesting that RA-dependent mechanisms are important in modifying the development of the skull, acting on the underlying mesectodermal developmental programmes that used to specify the reptilian skull.

There is more to come in this story but the information we have links development and the control of differentiation closely. An understanding of the methods by which the retinoids act may show how certain of their actions may be exploited in restoring control of the differentiated state in cells that have escaped from normal control mechanisms.

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