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Review article

Retinoic acid signalling in the development of the epidermis, the limbs and the secondary palate

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ABSTRACT

Retinoic acid (RA), the active derivative of vitamin A, is one of the major regulators of embryonic development, including the development of the epidermis, the limbs and the secondary palate. In the embryo, RA levels are tightly regulated by the activity of RA synthesizing and degrading enzymes. Aberrant RA levels due to genetic variations in RA metabolism pathways contribute to congenital malformations in these structures. *In vitro* and *in vivo* studies provide considerable evidence on the effects of RA and its possible role in the development of the epidermis, the limbs and the secondary palate. In conjunction with other regulatory factors, RA seems to stimulate the development of the epidermis by inducing proliferation and differentiation of ectodermal cells into epidermal cells. In the limbs, the exact timing of RA location and level is crucial to initiate limb bud formation and to allow chondrogenesis and subsequent osteogenesis. In the secondary palate, the correct RA concentration is a key factor for mesenchymal cell proliferation during palatal shelf outgrowth, elevation and adhesion, and finally to allow bone formation in the hard palate. These findings are highly relevant to understanding the mechanism of RA signalling in development and in the aetiology of specific congenital diseases.

Contents

1. Introduction

1.1. Vitamin A and its derivatives

Vitamin A is an essential micronutrient for embryonic development and growth in all vertebrate species (Clagett-Dame and Knutson, 2011; Duester, 2008; Zile, 2001). Either deficiency or excess of vitamin A outside its narrow optimal range is related to

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congenital malformations (Clagett-Dame and DeLuca, 2002; Ross et al., 2000). Vitamin A, more precisely, its active derivative retinoic acid (RA), is crucial for embryonic development from the very early stages on, since it regulates processes such as the establishment of the anterior-posterior and dorsal-ventral axes of the embryo through gradient formation (Diez del Corral and Storey, 2004; Molotkova et al., 2005; Paschaki et al., 2012; Wilson et al., 2004; Wilson and Maden, 2005). At the later stages of embryogenesis, RA is involved, among others, in the development of the epidermis, the limbs, and the secondary palate (Mark et al., 2006). Thus, RA is indispensable for embryonic development, and aberrant RA metabolism and signalling are involved in the aetiology of many congenital diseases.

The term "vitamin A" designates a family of related compounds possessing the biological activity of retinol (Blomhoff and Blomhoff, 2006). Natural and synthetic derivatives of retinol in the form of aldehydes or carboxylic acids are collectively called "retinoids", and are involved in a wide scale of biological processes. The carboxylic acid isomer all-*trans*-retinoic acid (RA) is a natural derivative of retinol and is one of the key regulators of gene expression during embryonic development (Duester, 2000; Mic et al., 2003). RA is an important regulator of gene expression during embryogenesis, and mutations in the genes involved in RA metabolism and/or signalling cause congenital defects (Lohnes et al., 1994; Saitou et al., 1995). The regulation of development of the epidermis, the limbs and the secondary palate includes many signalling molecules and/or pathways within the epithelium as well as interactions of these signals between the epithelium and the mesenchyme. The purpose of this review is to discuss the present knowledge on the role of RA in the development of these three structures.

We will first present a short overview of RA metabolism and signalling. Subsequently, we will discuss its role in the development of the epidermis, the limbs and the secondary palate.

2. Retinoic acid metabolism during embryonic development

Vitamin A cannot be synthesized by the human body neither in adulthood, nor during embryonic development (Blomhoff and Blomhoff, 2006). Maternal nutritional intake is therefore the only source of vitamin A during embryonic development. Two precursor forms of vitamin A are available in the human diet: retinyl palmitate (*i.e.* the esterified form of retinol) present in meat and carotenoids (β-carotene, α-carotene, and β-cryptoxantin) present in plants. Both dietary precursors of vitamin A are eventually converted into either retinoic acid (RA) or retinal, the functionally active derivatives of vitamin A, or are stored in the form of biologically inactive retinyl esters in the liver and to a lesser degree in adipose tissue (Schreiber et al., 2012).

The complex spatiotemporal regulatory functions of RA requires precise control of its distribution and concentration, which is achieved by RA-synthesizing and degrading enzymes. Embryonic RA metabolism and signalling are summarized in Fig. 1.[1]

The conversion of retinol into its active derivatives is mediated by a set of enzymes that are classified into three groups; the cytosolic alcohol dehydrogenases (ADHs) of the medium-chain dehydrogenase/reductase (MDR) superfamily, the microsomal retinol dehydrogenases (RDHs) of the short-chain dehydrogenase/reductase (SDR) superfamily and the retinaldehyde dehydrogenases (RALDHs) (Duester, 2000; Pares et al., 2008). RA is synthesized from retinol in two steps. Firstly, all-*trans* retinol is oxidized to all-*trans* retinal, which is subsequently oxidized to all-*trans* retinoic acid. The rate-limiting first step is mediated by the ADHs and the RDHs (Blomhoff and Blomhoff, 2006). ADH4 is the most active subtype of ADHs and the first one expressed in the craniofacial region, trunk and forelimb bud during early embryogenesis (Ang et al., 1996). RDH10 seems to be the most crucial member of the SDR superfamily for embryonic development, since loss of its function disrupts RA biosynthesis and signalling in the craniofacial and the trunk regions leading to orofacial, limb, and organ abnormalities and ultimately to death of the embryo (Sandell et al., 2007). The second step in RA biosynthesis is mediated by the RALDH subtypes 1, 2 and 3 in different organs and tissues (Maclean et al., 2009; Niederreither et al., 1999). RALDH1 is required for eye development and is also involved in the maintenance of adult vision (Fan et al., 2003), while RALDH3 is expressed in the embryonic craniofacial ectoderm. It is specifically present in the developing eye and nasal region (Mic et al., 2000) and, together with RALDH2, it is responsible for RA synthesis in the craniofacial structures (Halilagic et al., 2007; Okano et al., 2014). RALDH2 is the most essential RA synthesizing enzyme in the early mammalian embryo (Napoli, 2012). It is responsible for RA synthesis in most embryonic tissues including the maxillary process, the forelimb buds, and the dorsal spinal cord (Mic et al., 2002). RALDH1 and RALDH3 function later in embryonic development in specific organs such as the eye, the olfactory pit and the kidney (Duester, 2007). Once synthesized, RA is bound to cellular retinoic acid binding proteins (CRABP) I or II and is transported to the nucleus where it binds to nuclear receptors (RAR/RXR) and activates the expression of its target genes (Das et al., 2014). RA signal transduction will be discussed further in the next section.

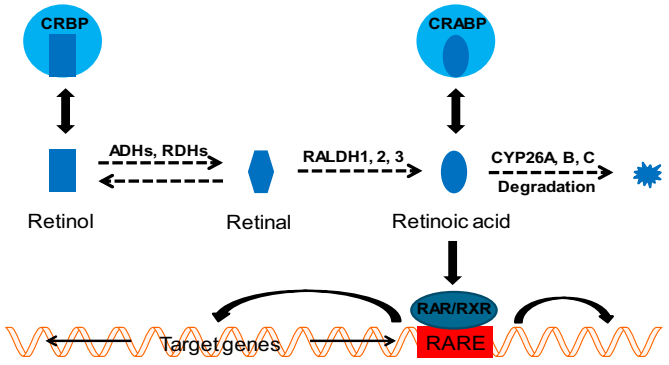

Fig. 1. Retinoic acid metabolism and signalling. After entering the cell, maternal retinol binds to cellular retinol-binding proteins (CRBP). Retinol is then oxidized to retinaldehyde by either alcohol dehydrogenases (ADHs) or retinol dehydrogenases (RDHs), and retinaldehyde is subsequently oxidized to RA by retinaldehyde dehydrogenases 1, 2 and 3 (RALDH1, 2, 3). Cellular RA-binding proteins (CRABP) facilitate the transport of RA to the nucleus. In the nucleus, RA acts as a ligand for RAR/RXR that can bind to retinoic acid response elements (RARE) in the regulatory regions of RA target genes. By activating the RAR/RXR-RARE complex, RA regulates the transcription of its target genes. RA can be degraded by cytochrome P450 family 26 (CYP26A, B, C).

RA can be degraded by enzymes of the cytochrome P450-26 subfamily (CYP26A1, CYP26B1, and CYP26C1). All three isotypes of CYP26 show spatiotemporal activity throughout embryonic development and hereby control endogenous RA concentrations. Cyp26A1 is the first RA degrading enzyme functioning during embryonic development followed by Cyp26C1 and Cyp26B1. While Cyp26A1 is expressed in the tailbud neuroepithelium and the cervical neural crest-derived mesenchyme, CYP26B1 is expressed in the developing limb bud and in the maxillary and mandibular processes. Cyp26C1 is only transiently expressed in the hindbrain (Loudig et al., 2005; MacLean et al., 2001; Tahayatoa et al., 2003; Yashiro et al., 2004).

Thus, RA is generated from maternal retinol in several steps and is active from the earliest stages of the embryogenesis. Its spatiotemporal distribution is achieved and is regulated by RA

[1] Adapted from (Ackermans et al., 2011).