

RESEARCH HIGHLIGHT

Opposing Biological Functions of Retinoic Acid in Normal Embryonic Development

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Vitamin A and its metabolic derivative, retinoic acid (RA) are essential biomolecules for normal development regulation and regeneration in different organs. Too much and deficiency of vitamin A can be toxic for fetuses lead to birth defects or is associated with many congenital malformations. It has been known that RA alone or in combination with other morphogens promotes the developmental program such as neural differentiation and patterning. Here, we discussed RA role in the neural patterning of the embryonic stem cells and its function in promoting the neural differentiation in neural plate of the developing embryo through attenuating the fibroblast growth factor (FGF) signaling. By using different techniques, we also argued the opposite function of RA in inducing apoptosis in the human umbilical cord-derived mesenchymal stem cells (hUCMSCs) shown by upregulating the caspase expression. Finally, we discussed that some biological parameters including cell density and passage appeared to be involved in this cytotoxicity response.

Keywords: Retinoids; Mesenchymal Stromal Cells; Embryonic Stem Cells; Growth and Development; Apoptosis

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Vitamin A (retinol), as a crucial dietary vitamin is an essential small lipophilic biomolecule for body homeostasis and plays a major role in vision, immune function, reproduction and development^[1]. The body cannot produce this vitamin de novo, so it is entered the small intestine as the retinoid and carotenoids forms and absorbed in enterocytes, wherein esterified as the retinyl esters for transport in the blood stream and storage in the hepatic stellate cells or hydrolyzed to retinol^[2]. The latter is bound to retinol binding protein (RBP) synthesized in liver and transported to the target cells where it is converted to retinoic acid via two consecutive enzymatic reactions. Firstly, retinol is oxidized to retinal by retinol dehydrogenase (RDH) and then converted to retinoic acid by retinaldehyde dehydrogenase (RALDH)^[2-4]. Retinoic acid (RA), as a biological morphogen has a pleiotropic effects and promotes proliferation,

apoptosis and differentiation in normal embryonic development and regenerates many organs such as lens, limb, central nervous system, lung and hematopoietic tissues. These functions of RA have been shown to be mediated through two classes of receptors, RAR and RXR binding to retinoic acid response elements (RAREs) found in genes involving in retinoid signaling pathway^[5-11].

Using a range of vertebrate models and different stem cells including embryonic and fetal stem cells, multiple biological roles of RA have been studied. These studies have discovered the key functions of RA in embryonic development, ranging from the early axial patterning of the embryo and control of neurogenesis to many roles in cell proliferation and apoptosis. A growing number of literatures stated bone morphogenetic proteins (BMP), sonic hedgehog

(SHH), Wnt, and fibroblast growth factors (FGF) as well as RA are potent morphogens promoting the neural fate of the developing neuron in neural tube toward the interneurons and motor neurons, spatiotemporally. Recently, we indicated this morphogenic role of RA in neural patterning and regional identity in mouse embryonic stem cells isolated from mouse blastocyst and directs their differentiation toward the dorsal and intermediate interneurons as well as motor neurons with hindbrain and cervical spinal cord identities ^[12]. Interestingly, we also revealed that RA mimics the embryonic somite functions to promote neural differentiation in developing caudal neural plate (CNP) located at the caudal end of the developing embryo (Unpublished Data). Previous studies have shown that the epiblast cells around the embryonic primitive streak have a dual fate, either penetrate beneath the CNP to create paraxial mesoderm (somite) forming ribs, vertebrae and muscle or reside in CNP to become neural precursor cell. It has been shown that FGF signaling has a major role in this decision and RA secreting from embryonic somite converts the neural precursor cells to the differentiated neurons through attenuating the FGF signaling ^[13-15].

In contrast to neural differentiation role of RA in previous described studies, our recently published work addressed the opposite function for RA on fetal cells that it promotes apoptosis and no differentiation on human umbilical cord-derived mesenchymal stem cells (hUCMSCs) ^[16]. Apoptosis is the process of programmed cell death which occurs in normal physiological condition such as cell turnover, immunity system regulation and embryogenesis. It can be triggered by physical and chemical stimuli through intrinsic (mitochondrial pathway) or extrinsic (death receptor pathway) mechanisms involved by the caspase enzyme actions ^[17]. Among the different protocols, MTT (3-(4,5-dimethylthiazol-2-yl) diphenyl tetrazolium bromide) and TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling) assays as well as AO/EB (acridine orange/ethidium bromide) staining are widely used methods for cell viability evaluation. MTT is a salt that is cleaved into a bluish product termed formazan in living cells, by the mitochondrial enzyme succinate dehydrogenase. In addition, TUNEL is an assay that detects the fragmented DNA in apoptotic cells ^[18]. Moreover, AO/EB are stains that illustrate the apoptotic body formation and intercalate into DNA to visualize the nuclear changes in injured cells ^[19]. By using these techniques, we attempted to describe that RA in a concentration-dependent manner induces apoptosis in hUCMSCs through upregulation of Caspase 3/8/9 expression and increasing Bax/Bcl2 ratio, suggesting the involvement of both extrinsic and intrinsic apoptotic pathways in RA-treated cells. We also found the cytoplasmic blebbing and nuclear morphology changes in hUCMSCs, proposing the early to

late stages of apoptosis in damaged cells. In this work, we argued that some biological parameters including initial time seeding, cell density, passage number and exposure time of RA treatment affect the hUCMSCs cytotoxic response. The cells initially cultured for 72h (more than one doubling time) and at low density revealed more sensitivity to RA and among different passages (P), the cells at P2 represented higher cytotoxicity response. These findings propose the importance of logarithmic phase of the growth kinetics in cellular response to cytotoxic agents such as RA. This was confirmed by the telomerase activity assay in hUCMSCs at P2 found the higher telomerase activity rate than the other passages ^[16].

In recent years, an increased number of researchers have been interested to elucidate in vivo and in vitro teratogenic effects of RA on different cells and organs at cellular and molecular levels, since the retinoid teratogenicity in embryo resulted in congenital malformation in many organs including skeletal and neural crest-derived structures ^[20-22]. Too much vitamin A can be toxic for fetuses and causes to retarded embryonic development and impacts on offspring homeostasis. On the other hand, deficiency of RA is lead to anomalies in cardiac development ^[23] and craniofacial structures ^[24]. Therefore, the correct balance of RA should be maintained for normal development and regeneration in embryo.

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