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Mini-review

Aldehyde dehydrogenases and cancer stem cells

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ABSTRACT

Aldehyde dehydrogenases (ALDHs), as essential regulators of aldehyde metabolism in the human body, protect organisms from damage induced by active aldehydes. Given their roles in different cancer types, ALDHs have been evaluated as potential prognostic markers of cancer. ALDHs exhibit high activity in cancer stem cells (CSCs) and may serve as markers of CSCs. Moreover, studies indicated that ALDHs and their regulated retinoic acid, reactive oxygen species and reactive aldehydes metabolism were strongly related with various properties of CSCs. Besides, recent research evidences have demonstrated the transcriptional and post-translational regulation of ALDH expression and activation in CSCs. Thus, this review focuses on the function and regulation of ALDHs in CSCs, particularly ALDH1A1 and ALDH1A3.

Introduction

Aldehyde dehydrogenases (ALDHs), broadly defined as a superfamily of NADP(+)-dependent enzymes, participate in aldehyde metabolism, catalysing the oxidation of exogenous aldehydes (drugs and ethanol) and endogenous aldehydes (lipid, amino acids, or vitamins) into their corresponding carboxylic acids [1]. The primary toxicity of aldehydes can induce enzyme inactivation, DNA damage, impaired cellular homeostasis and even cell death by forming adducts with various cellular targets including glutathione, nucleic acids and amino acids [2,3]. Deficiency and polymorphisms of *ALDHs* in organisms are related to diseases such as Parkinson's disease, Type II hyperprolinaemia, hypertension and Sjögren–Larsson syndrome, and may even contribute to the occurrence of carcinoma [4–8]. Numerous studies have indicated that tumours with high malignancy have high levels of ALDHs [9,10].

Cancer stem cells (CSCs) may have been first identified in teratocarcinomas [11,12], with its initial clues date back to the 19th century [13]. Kleinsmith and Pierce [12] established the immortal pluripotent teratocarcinoma lines from a single transplanted multipotent malignant cell, strongly suggesting the existence of CSCs. Further data demonstrated the existence of CSCs in leukaemia and multiple solid tumours [14–16]. CSCs, also called tumour-initiating cells, comprise a small distinct subpopulation of tumour cells which possess high self-renewal properties, multiple differentiation capacity, tumourigenesis and drug resistance. The theory of cancer stem cell proposes an attractive cellular mechanism for the current unsatisfactory treatments. However, since the first discovery, challenges have arisen on how to effectively target CSCs. Recent studies have exhibited the importance of metabolic reprogramming as the hallmark of cancer and a growing number of results have established a link between material metabolisms and CSCs. For instance, the metabolic enzyme glycine decarboxylase, which functions in glycine metabolism, drive the tumorigenicity of CSCs in non-small cell lung cancer (NSCLC) [17]. Mutations in metabolic enzymes such as isocitrate dehydrogenase-2 play multiple roles in leukaemia initiation and maintenance [18]. As important metabolic enzymes in CSCs, ALDHs and their metabolic substrates retinoic acid (RA), reactive oxygen species (ROS) and reactive aldehydes directly and indirectly influence the various cellular processes in CSCs; these processes include target gene expression, protein translation and signal transduction. Moreover, ALDHs are being widely used to isolate and identify various CSCs and are regarded as consistent CSC markers [19], compared with other CSC surface markers, such as CD24, CD44, CD133, CD166 and epithelial cell adhesion molecule, which are limited to specific types of tumours [20,21].

ALDHs have vital roles as metabolic enzymes and universal markers in CSCs. Accumulating evidence on the functional role of ALDHs in CSCs is available; however, the specific mechanisms involved in the regulation of ALDHs in CSCs remain unclear. Thus, this review focuses on the biological effects of ALDHs and the mechanisms underlying ALDH regulation in CSCs and provides insights into the potential therapeutic applications of ALDHs in CSC elimination.

ALDH family

The following 19 ALDH subtypes with various chromosome locations have been detected in humans: *1A1*, *1A2*, *1A3*, *1B1*, *1L1*, *1L2*, *2*, *3A1*, *3A2*, *3B1*, *3B2*, *4A1*, *5A1*, *6A1*, *7A1*, *8A1*, *9A1*, *16A1* and *18A1*

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[22,23]. Information on ALDHs is also available online (http://www.aldh.org). Alternatively spliced transcriptional variants exist in most of the 19 human ALDH genes enumerated above; however, their function and significance remain to be established. ALDHs have 11 families and 4 subfamilies, which are distributed in various cellular compartments, including cytoplasm, nucleus, mitochondria and endoplasmic reticulum [24]. Most ALDH isoforms are widely distributed in the body, with the highest concentrations in the liver and kidney [24].

Various ALDHs are considered to have specific biological roles aside from acting as enzymes which eliminate toxic biogenic and xenobiotic aldehydes. For instance, the ALDH1 family plays a vital role in RA signalling, which is essential for embryogenesis and development [25]. ALDH2 takes part in acetaldehyde detoxification and is significantly correlated with alcohol-mediated tumours [26]. ALDH1A1 and ALDH3A1 are lens and corneal crystallins which protect against ultraviolet (UV) radiation-induced damage [27]. ALDH7A1 involves in the pipecolic acid pathway of lysine catabolism, which can regulate osmotic pressure and has been recently implicated in prostate cancer metastasis [28].

ALDHs and cancer

ALDHs have been recently regarded as potential novel cancer prognostic markers. Studies on gastric cancer have found that ALDH1A1 overexpression was closely related to poor prognosis in patient subgroups stratified by tumour size, depth invasion and lymph node metastasis. Patients with ALDH1A1 overexpression have poor overall survival and short recurrence-free survival [29,30]. Similar studies have associated ALDH1-positive tumours with poor clinical prognosis in breast, lung, pancreatic and prostate cancers, as well as in head and neck squamous cell carcinomas [31–36].

ALDHs also act as opposite roles in the initiation and development of carcinoma. For example, ALDH2, as a key enzyme which oxidises acetaldehyde, participates in alcohol metabolism and is associated with alcohol-mediated carcinogenesis [37]. It has been reported that up to 8% of the world's population carries a dominant-negative mutation in *ALDH2*. Alcohol consumption in these individuals has showed a good correlation with the risk for developing upper gastrointestinal tumour, particularly oesophageal cancer [38,39]. Another study found that the acetaldehyde-catabolising enzyme Aldh2 is essential for the development of *Fancd2−/−* embryos and that *Aldh2−/−Fancd2−/−* mice have a high risk for the spontaneous development of acute leukaemia [40]. Other isozymes such as ALDH1A2, a regulator of RA synthesis in developing tissues, were discovered as candidate tumour suppressors in prostate cancer [41]. However, ALDH1A1 was found as a transcription target of the leukaemogenic factor TLX1/HOX1 and may induce tumour growth in leukaemia [42].

ALDHs and CSCs

ALDHs play critical roles in normal stem cell functions during development [43]. Recent studies have linked potent ALDH activity, which was detected using a quantified commercial assay known as Aldefluor assay, to CSC isolation and identification [44]. van den Hoogen et al. [45] evaluated ALDH-high prostate cancer cells by using Aldefluor assay and found that this population of cells displays strongly elevated clonogenicity and migratory behaviour in vitro. Further studies discovered that this subpopulation of cells readily forms distant metastases with strongly enhanced tumour progression at both orthotopic and metastatic sites in a nude mouse model [45]. Shao et al. [46] reported that ALDH1A3 isozyme is a marker for a subpopulation of highly clonogenic and tumourigenic NSCLC cells and that STAT3 activation is essential to maintain the subpopulation of ALDH1-positive lung cancer cells. Other studies which used the same sorting methods demonstrated that bladder cancer cells and cervical cancer cells with high ALDH activity display CSC functions, including high tumourigenicity, enhanced self-renewal and differentiation potentials [10,47].

ALDH1A1 and ALDH3A1 are not the only isozymes responsible for Aldefluor activity; other ALDH isoforms such as ALDH1B1, ALDH1A7 and ALDH7A1 also exhibit elevated expression in CSCs [48]. However, the role of these isozymes in CSCs remains unsupported by compelling evidence. Therefore, ALDH1A1 and ALDH3A1 are the commonly used markers for most CSCs. These markers also play important roles in regulating critical processes in CSCs.

Functional roles of ALDHs in CSCs

The precise mechanism underlying the effects of ALDHs in CSC maintenance has yet to be clarified. However, ALDHs and their regulated retinoic acid, reactive oxygen species and reactive aldehydes metabolism likely contribute to its functional roles.

Role in RA-mediated signalling pathways

RA signalling plays significant roles in embryonic stem cells [49] and tumour cells [50]. The anti-tumour activity of RA is due to the activation of a series of cellular genetic programs which modulate cell differentiation, apoptosis and growth [51], involved in the classical pathway. Through this pathway (Fig. 1), retinol (vitamin A) absorbed by cells is oxidised to retinal by retinol dehydrogenases. Then, retinal is oxidised to RA in a reaction catalysed by ALDH1A1, ALDH1A2, ALDH1A3 and ALDH8A1. The metabolised product RA includes all-trans RA (ATRA), 9-*cis* RA and 13-*cis* RA. The ALDH isoforms, especially ALDH1A1, have high affinity to ATRA and 9-*cis* RA. RA can enter the nucleus and induce the transcriptional activity of downstream effectors through the activation of heterodimers of RA receptors (RAR-α, β, γ) and retinoic X receptors (RXR-α, β, γ). The role of RA signalling in CSCs has been reported recently. Ginestier et al. [52] have shown that ALDH regulates breast CSC biology by affecting retinoid metabolism; retinoid signalling modulation may be sufficient to induce the differentiation of breast CSCs. RA can bind to its nuclear receptors (RARα or RARβ) and activate gene expression related to loss of stem cell markers, differentiation, cell cycle arrest and morphology change; these phenomena eventually reduce tumour propagation and inhibit tumour growth [53]. The subsequent up-regulation of these receptors generates a positive feedback loop for RA signalling. Moreover, the Notch pathway is the downstream target of retinoids. Constitutive activation of Notch signalling rescues RA-induced differentiation and stem cell depletion [53].

Given their capabilities to induce cellular differentiation, apoptosis and cell cycle arrest, ATRA, 9-*cis* RA and 13-*cis* RA have been extensively investigated for their roles in cancer treatment [54]. ATRA was approved by the Food and Drug Administration of America for the treatment of acute promyelocytic leukaemia (APL) [55] in 1995. The advent of ATRA and its combination with chemotherapy contributed in the past decades to optimise the anti-leukaemic efficacy in acute APL, leading to complete remission rates greater than 90% [56]. However, the therapeutic effect of ATRA in solid cancer remains controversial. Trials investigating RA in breast cancer, lung cancer, and head and neck cancer have shown negative responses [57–60]. Several potential mechanisms, especially the non-classical pathways, are proposed for the less successful therapeutic effect of RA in solid tumour.

RA and its receptors can participate in other important pathways, also referred to as non-classical pathways, aside from the classical pathway mentioned above. The non-classical pathways,

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