Chapter 1

Aldehyde Dehydrogenase: Cancer and Stem Cells

Adil M. Allahverdiyev, Malahat Bagirova, Olga Nehir Oztel, Serkan Yaman, Emrah Sefik Abamor, Rabia Cakir Koc, Sezen Canim Ates, Serhat Elcicek and Serap Yesilkir Baydar

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1. Introduction

Aldehyde dehydrogenases (ALDH) belong to the oxidoreductase family, which catalyze the conversion of aldehydes to their corresponding acids. As a group of NAD(P)+-dependent enzymes, aldehyde dehydrogenases (ALDHs) are involved in oxidation of a large number of aldehydes into their weak carboxylic acids (Moreb, *et al.*, 2012). ALDH is found in every subcellular region such as cytosol, endoplasmic reticulum, mitochondria, and the nucleus, with some even found in more than one location (Marchitti, *et al.*, 2008).

ALDH is also found in stem cells. During early life and growth, stem cells (SCs) have a spectacular potential to develop into several cell types in the body. In many tissues, SCs behave as a kind of internal repair system, dividing essentially without limit to replenish other cells (Fuchs & Segre, 2000). Stem cells are distinguished from other cell types by two important characteristics: (1) Their unspecialized properties and renewal potencies; and (2) differentiation into other cell types under certain physiologic or experimental conditions (Discher, *et al.*, 2009, Solis, *et al.*, 2012). These cells are identified by their expression of a particular panel of surface molecules, with the presence of CD73, CD90, CD105, and the absence of CD14, CD34, CD45, and HLA-DR. They show no proliferative response from alloreactive lymphocytes because of the negligible levels of extracellular MHC class I and II determinants and they also have important immunomodulatory functions in all the cells involved in both the innate and adaptive immune responses (Nauta & Fibbe, 2007). On the other hand, cancer stem cell theory is supported by biological reason for aging. The theory postulates that cancer SCs, a small subset of tumor cells also have stem cell-like properties (epithelial-to-mesenchymal progression, differentiation and self-renewing capacity). ALDH expression has demonstrated itself to be a possibly relevant prognostic marker. For this

reason, the subpopulation of cancer SCs (CSCs) can present a therapeutic target for poor-prognostic, treatment-resistant and recurrent breast cancer. Through its role in oxidizing retinol to RA, which is a modulator of cell proliferation, ALDH1 might have a role in early differentiation of SCs and stem cell proliferation (Mieog, *et al.*, 2012).

There are several isoforms of ALDH (ALDH1A1, ALDH1A2, ALDH1A3 and ALDH8A1) that play a role in RA formation by oxidation of all-trans-retinal and 9-cis-retinal in RA cell signaling, which has been related to the "stemness" characteristics of SCs (Marcato, *et al.*, 2011). ALDH1 is better as a marker of breast cancer SCs than CD44+/CD24- (Tanei, *et al.*, 2009). While cellular markers including CD133 have been used to identify tumor SCs, especially for glioblastomas (GBMs) ALDH1 was desrcribed as a marker for the identification of non-neoplastic SCs and tumor stem cells (TSCs) (Corti, *et al.*, 2006, Ginestier, *et al.*, 2007, Huang, *et al.*, 2009).

After CD133- GBMs are characterized to behave as brain TSCs(Beier, *et al.*, 2007). ALDH1 has also been described as a stem cell marker in various solid neoplasms including lung cancer (Jiang, *et al.*, 2009), breast carcinoma (Ginestier, *et al.*, 2007), colorectal cancer (Huang, *et al.*, 2009), and GBM. ALDH1B1 and ALDH1A1 are differentially expressed in normal human tissues. ALDH1B1 is expressed at higher levels than ALDH1A1 in human epithelial cancers. ALDH1B1 was abundantly expressed in adenocarcinomas originating from the tissue and particularly in colonic adenocarcinoma (Chen, *et al.*, 2011).

$ALDH^{br}$ cells can be detected with ALDEFLUOR reagent by using flow cytometry or fluorescent microscopy. Aldefluor assay is based on the conversion of fluorescent non-toxic substrate for ALDH substrate to the fluorescent reaction product. Non-toxic substrate for ALDH can freely diffuse into intact, viable cells. The BODIPY aminoacetaldehyde is converted to the fluorescent product BODIPY aminoacetate by ALDH activity. These cell populations, which are known as ALDH bright ($ALDH^{br}$) cells are isolated from adult tissues by flow sorting. $ALDH^{br}$ cells were also found in various cancer tissues including breast, liver, colon, pancreas, prostate, lung, ovarian and acute myelogenous leukemia and are related to cancer chemo resistance (Siclari & Qin, 2010).

$ALDH^{br}$ population may play an important role in regenerative medicine. The regenerative potential of $ALDH^{br}$ cells obtained from different tissues was investigated in various disease models such as ischemic tissue damage, hind limb model, brain damage (spinal motor atrophy, etc.) and pancreatitis.

Thus, as mentioned above, ALDH is an important enzyme for cancer and stem cells. This chapter aims to represent the important role of aldehyde dehydrogenases in stem cells, cancer stem cells, therapy and regenerative medicine.

1.1. Aldehydes

Aldehydes are formed in various physiological processes such as catabolism of transmitters like GABA, serotonin, adrenaline, noradrenaline and dopamine, as well as catabolism of amino acids. In addition, there are more than 200 different aldehydes that are produced

through lipid, and aldehydic intermediates through carbohydrate metabolism. Along with these endogenous aldehydes, there are also exogenously present aldehydes in a variety of industrial processes, including the production of polyester plastics (formaldehyde, acetaldehyde, acrolein, etc.), polyurethane, smog, cigarette smoke or motor vehicle exhaust. With their malodorous properties, some dietary and aromatic aldehydes are accepted as additives in food and cosmetics (e.g., citral, cinnamaldehyde, benzaldehyde, and retinal), though many others are cytotoxic (Chen, *et al.*, 2010). Aldehydes could interact with thiol compounds of some proteins, leading to structural and functional alterations of these molecules (Weiner, *et al.*, 2008). In order to protect the human body from the deleterious effects of aldehydes in general, and myocardium and the brain in particular, a fast aldehyde detoxification mechanism is essential. Aldehydes are significantly reactive and possess high diffusion capacities in cells, thus they can easily form complexes with DNA, proteins and lipids, of which they can alter the function and cause their inactivation. As a result of DNA damage induced by these complexes, many aldehydes are classified as mutagenic or carcinogenic, including acetaldehyde, which is derived from ethanol consumption. Over–consumption of ethanol has been related to liver disease and several gastrointestinal and upper aerodigestive cancers. Numerous other cytotoxic and reactive aldehydes have been shown to be linked with other types of diseases (Hofseth & Wargovich, 2007, Perluigi, *et al.*, 2009, Chen, *et al.*, 2010).

1.2. Aldehyde dehydrogenases

Aldehyde dehydrogenases [EC 1.2.1.3; *systematic name*: aldehyde: NAD(P)$^+$oxidoreductase] catalyze aldehyde conversion into their matching acids by NAD(P)$^+$-dependent nearly irreversible reaction. In 1949, mammalian ALDH was first discovered in ox liver. After that, many types of ALDH were distinguished according to their physico-chemical characteristics, enzymological properties, subcellular localization, and tissue distribution (Oraldi, *et al.*, 2011). They are involved in several cell functions such as proliferation, differentiation, survival as well as cellular response to oxidative stress (Jackson, *et al.*, 2011). ALDHs are commonly delivered from bacteria and humans (Moreb, 2008). Based on their physico-chemical characteristics, subcellular localization, tissue distribution and enzymological properties, a number of types of ALDH have been distinguished since the 1960s, around the time when mammalian ALDH activity was observed in ox for the first time. In 1985, 2 ALDH genes were cloned and characterized. Genes or cDNAs for more than 50 animals, fungi and bacterial ALDHs in addition to protein sequences have been discovered (Yoshida, *et al.*, 1998).

The human genome contains 19 ALDH functional genes and 3 pseudogenes (Black, *et al.*, 2009). At least 5 ALDH isozymes function in the mitochondria, and all the ALDH genes are encoded in the nucleus (Chen, *et al.*, 2010).

All of the ALDH gene superfamily plays an important role in the enzymic detoxification of endogenous and exogenous aldehydes. They are also involved in the formation of molecules that are important in cellular processes like RA, betaine and gamma aminobutyric acid

formation. Furthermore, ALDHs also have several non-enzymic functions such as binding to some hormones and other small molecules and decreasing the effects of ultraviolet irradiation in the cornea (Pappa, *et al.*, 2003, Wymore, *et al.*, 2004). The most important role of ALDHs is detoxification of aldehydes, which caused cytotoxicity, mutagenicity, genotoxicity, and carcinogenesis in healthy cells. Mutations in ALDH genes cause severe diseases including Sjögren-Larsson syndrome, pyridoxine-dependent seizures, and type II hyperprolinemia, and also plays a role in cancer and Alzheimer's disease (Black, *et al.*, 2009).

Functions of some of these ALDHs in endobiotic and xenobiotic metabolisms have been highly reviewed before and the distinctive metabolic pathways' influences have been depicted. Because of their chemical reactivity, many distinct aldehydes are pervasive in nature and are toxic at low levels. Hence, levels of metabolic-intermediate aldehydes should be cautiously regulated. The presence of several distinct ALDH families in most studied organisms seem to have wide fundamental tissue distribution. A wide range of allelic variants within the ALDH gene family have been identified, leading to heterogeneity in pharmacogenetic characteristics between individuals, resulting distinctive phenotypes including intolerance to alcohol and increased risk of ethanol-induced cancers in most cases (ALDH2 and ALDH1A1), Sjogren-Larson Syndrome (ALDH3A1), type II hyperprolinemia (ALDH4A1), 4-hydroxybutyric aciduria, mental retardation and seizures (ALDH5A1), developmental delay (ALDH6A1), hyperammonemia (ALDH18A1), Pyridoxine-dependent epilepsy (ALDH7A1), and late-onset Alzheimer's disease (ALDH2).

ALDH dysfunction could also be caused by drugs and environmental substances, substrate inhibition, as well as oxidative and metabolic stress. ALDH activity in drug resistance to oxazaphosphorines is one of the most vigorously studied pathways. The role of ALDH1A1 in drug resistance has been studied first in hematopoietic progenitors and more recently in lung cancer (Marchitti, *et al.*, 2008).

2. Stem cells

During early life and growth, SCs have a spectacular potential to develop into several cell types in the body. In many tissues, SCs behave as a kind of internal repair system, dividing essentially without limit to replenish other cells (Weissman, 2000). Stem cells are distinguished from other cell types by two important characteristics: First, they are unspecialized cells and, sometimes after long periods of inactivity, they can renew themselves through cell division; second, under certain physiologic or experimental conditions, they are naturally sensitive to their environment, responding to chemical, physical, and mechanical features of their matrices or substrates (Discher, *et al.*, 2009, Solis, *et al.*, 2012).

Until recently, scientists primarily worked with two kinds of SCs from animals and humans: embryonic SCs and non-embryonic "somatic" or "adult" SCs (Feng, *et al.*, 2009).

In 1981, scientists discovered ways to derive embryonic SCs from mouse embryos. In 1998, a detailed study of the biology of mouse SCs led to the discovery of a method to derive SCs

from human embryos and grow the cells in the laboratory, and these cells are called human embryonic SCs.

In 2006, genetically "reprogrammed" stem-cell-like cells were identified by using specialized adult cells. This new type of stem cell is called **induced pluripotent SCs (iPSCs)** (Krishna, *et al.*, 2011).

2.1. Cancer stem cells

Cancer is a class of diseases characterized by unregulated cell growth(Deisboeck, *et al.*, 2011). Cancer initiation depends on genetic mutations in series that affects cellular programming. Many cancer researches have focused on the identification and characterization of these genetic and molecular properties of cancer cells (Balmain, *et al.*, 2003). Tumors are also heterogeneous cellular entities whose growth is dependent upon dynamic interactions among the cancer cells themselves, and between cells and the constantly changing microenvironment (Bissell & Radisky, 2001). That kind of interaction is depent on signaling through cell adhesion molecules and different cell responses to growth factors and other external signals. All of these interactive processes act together to control cell phenotypic behaviors such as proliferation, apoptosis, and migration. There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected (Lakshmi Prasanna & Sathish Kumar, 2011).

According to recent statistics, cancer accounts for about 23% of the total deaths in the USA and is the second most common cause of death after heart disease (Jemal, *et al.*, 2007).

Cancer is caused by many internal and external factors. Inherited mutations, hormones, and immune conditions are internal factors while tobacco, diet, radiation, and infectious organisms are environmental/acquired factors (Kalluri & Weinberg, 2009, Nagy, *et al.*, 2010, Langley & Fidler, 2011, Mantel & Schmidt-Weber, 2011, Noman, *et al.*, 2011). In recent years, a particular sub-population of tumor cells are said to have a critical role in cancer; these cells are commonly called **CSCs** or **tumor initiating cells (TICs).** In most cancer types, CScs have been identified. CSCs are characterised by their two important properties: (1) Enhanced tumorigenicity; and (2) the capacity for self-renewal/differentiation (Bonnet & Dick, 1997, Al-Hajj, *et al.*, 2003). Thus, isolating CSCs is important in analyzing their characteristics *in vitro*. The isolated CSC population will not only give rise to de novo tumors with high efficiency, but will also recapitulate the tumor with both CSC and non-CSC populations.

One potential human CSC marker is the membrane antigen CD133 (Prominin) identified in subpopulations of cells in brain, colon and lung tumors (Singh, *et al.*, 2004, Ricci-Vitiani, *et al.*, 2007, Eramo, *et al.*, 2008). CD133+ tumor cells are also a marker identifying lung CSCs (Wang, *et al.*, 2008, Salnikov, *et al.*, 2010).

The expression and activity of ALDHs is determined as another potential CSC marker (Ginestier, *et al.*, 2007). ALDH1 is a marker of normal and malignant human mammary SCs and a predictor of poor clinical outcome (Huang, *et al.*, 2009). Aldehyde dehydrogenase

enzymes participate in cellular detoxification, differentiation and drug resistance through the oxidation of cellular aldehydes (Moreb, *et al.*, 1996).

The functional activity of ALDH has been widely used to identify and isolate CSCs found in the bone marrow (Ran, *et al.*, 2009), breast (Ginestier, *et al.*, 2007), lung (Ucar, *et al.*, 2009), ovary (Deng, *et al.*, 2010), colon (Huang, *et al.*, 2009), prostate (van den Hoogen, *et al.*, 2010), and pancreas (Dembinski & Krauss, 2009).

2.2. Stem cell markers

The derivation of SCs from adult tissues, their relative ease of isolation and enormous expansion potential in culture make them attractive therapeutic candidates (Prockop, *et al.*, 2010). These cells are identified by their expression of a particular panel of surface molecules, with the presence of CD73, CD90, CD105, and the absence of CD14, CD34, CD45, and HLA-DR. They show no proliferative response from alloreactive lymphocytes because of the negligible levels of extracellular MHC class I and II determinants. SCs also have important immunomodulatory functions in all the cells involved in both the innate and adaptive immune responses (Nauta & Fibbe, 2007).

3. ALDH as a stem cell marker

In theory, ALDH isozymes including ALDH1A, ALDH1A2, ALDH1A3, and ALDH3A1, which are involved in drug resistance and RA formation, are vital in protecting SCs against toxic endogenous and exogenous aldehydes and for SCs' ability to differentiate, respectively. It is unknown what ALDH isozymes are responsible for the ALDH activity that are used to identify stem cell progenitors. In the overlap gene profile of different stem cell populations, ALDH7A1, known as antiquitin, and ALDH2 were identified, consequently, and are worthy of further investigation. There is more about ALDH to be explored as a cause of its full physiological function has remained elusive. ALDH7A1 is a green pea 26g protein, which has function in regulation of turgor pressure, and has ≥50% amino acid identity with the 3 pseudogenes in the ALDH family. It also has 69% equity with ALDH2, but nevertheless has considerably lower affinity for acetaldehyde than ALDH2. However, ALDH2, which is a mitochondrial enzyme, has been widely studied mostly for its affilitation with ethanol metabolism. Yet, there might be an extent of confusion as to how ALDH2 is associated in gene profiling studies. According to the nomenclature, this enzyme indeed is ALDH1A, related to a series of events linked to the development of dopaminergic neurons through its ability to produce RA. It was reported that ALDH2 or AHD2 expression changes during differentiation of NIH-3T3 cells into adipocytes. These studies continue to focus on ALDH1A1's role in SCs and stem cell differentiation. For hematopoietic stem cell progenitors, ALDH1A1 has been a thoroughly established marker for many years. Reseach on the role of RA in granulocyte differentiation of hematopoietic SCs discovered that ALDH1A1 and ALDH1B1 catalyze cellular RA synthesis and are expressed in CD34+ hematopoietic progenitors (Russo, *et al.*, 2002, Luo, *et al.*, 2007).They also showed that ALDH1A2 or 1A3 do not show those characteristics. For the differentiation to mature

granulocytes, these 2 enzymes' expressions are necessary, however their expressions are lost once the differentiation is complete. The *in vitro* disulfiram treatment in which disulfiram acts as an ALDH inhibitor may inhibit granulocytic differentiation. ALDH1A1 is found in erythrocytes and has been pointed to contribute to the aldophosphamide detoxification. A study that inhibited ALDH and retinoid signaling with diethylamino-benzaldehyde (DEAB) that reported the expansion of human HSCs probably by blocking differentiation and assisting self-renewal and HSC expansion (Marchitti, *et al*., 2008).

The cancer stem cell theory is supported by current evidence in tumor biology, which may also provide a biological reason for the age-related survival difference. The theory demonstrates that CSCs, a small subset of tumor cells with stem cell-like properties such as epithelial-to-mesenchymal progression, are capable of differentiation and self-renewal, after which leads to formation of a heterogeneous tumor cell population. Including aldehyde dehydrogenase-1 (ALDH1) activity, CD44+/CD24-, CD133, and ITGA6, a wide range of putative breast cancer stem cell markers have been proposed. ALDH1 expression has especially demonstrated an assurance of a clinically relevant prognostic marker. In addition, the subset of CSCs is shown to be relatively insusceptible to chemo and radiotherapy by various studies. For this reason, the subpopulation of CSCs can present a statement and a therapeutic target for poor-prognostic, treatment-resistant and recurrent breast cancer. Through its role in oxidizing retinol to RA, which is a modulator of cell proliferation, ALDH1 might have a role in early differentiation of SCs and stem cell proliferation (Mieog, *et al*., 2012).

It is possible to isolate leukemia SCs depending on the elevated ALDH activity by using the aldefluor assay. In patient samples, the researchers encountered a population of ALDH+ acute myeloid leukemia (AML) cells (Rollins-Raval, *et al*., 2012). In most cases, the ALDH+ AML cells coexpressed CD34+ (formerly determined leukemia stem cell marker), and were introduced considerably better than the ALDH- AML cells in immunocompromised mice. In the same year, ALDH+ cells from breast cancers, which had the tumorigenic and self-renewal features of CSCs, were shown to be possibly isolated. This innovative study displayed the potential applicability of quantifying ALDH activity in solid tumors. ALDH activity would be used successfully as a CSC marker for abundant cancers including liver, colon, lung, bone, prostate, pancreatic, head and neck, thyroid, bladder, brain, cervical and melanoma in the proceeding years. With one exception of a current study for melanoma, 35 demonstrate growing evidence recommending ALDH's activity to be a universal CSC marker. Nonetheless, as amounted by the aldefluor assay in various tissues and cancers, the cause of ALDH activity may differ. Essentially, determination of specific ALDH isoforms carried out commonly in certain cancers might have prognostic suitability. Besides their valuable function in detoxification of aldehydes, ALDHs carry out other functions such as serving as binding proteins for various molecules (e.g., androgens and cholestorol), potentially act as antioxidants by NAD(P)H production, ultraviolet light absorption and/or hydroxyl radical scavenging and ester hydrolysis.

Lastly, several isoforms (ALDH1A1, ALDH1A2, ALDH1A3 and ALDH8A1), take place via RA formation by oxidation of all-trans-retinal and 9-cis-retinal in RA cell signaling, which

has been related to the "stemness" characteristics of CSCs. Consequently, its supported by widening evidence that ALDH may be more than just a CSC marker and have an accomplishable role in CSC biology (Marcato, *et al.*, 2011).

3.1. ALDH family members as stem cell markers

ALDH proteins can be found in every subcellular region such as cytosol, endoplasmic reticulum, mitochondria, and the nucleus, with some even found in more than one location. ALDH isozymes found in organelles besides cytosol carry signal or leader sequences that make their translocation to specific subcellular regions possible. After translocation or import, while nuclear and microsomal signals remain intact, mitochondrial sequences might be removed (causing mature proteins to be shorter). Most of the ALDHs have a large tissue distribution and show distinct substrate specificity (Marchitti, *et al.*, 2008).

3.1.1. ALDH1A1

ALDH1A1 encodes a homotetramer that is ubiquitously distributed in the adult epithelia of several organs such as brain, testis, kidney, eye lens, retina, liver and lungs. ALDH1A1 takes its position among the three highly-conserved cytosolic isozymes (see ALDH1A2 and ALDH1A3), which catalyze the oxidation of the retinol metabolite, retinal (retinaldehyde), to RA. ALDH1A1 has great affinity for the oxidation of both all-*trans*-(K_m < 0.1 μM) and 9-*cis*-retinal. By serving as a ligand for nuclear RA receptors (RAR) and retinoid X receptors (RXR), RA regulates gene expression; therefore its synthesis is crucial for normal growth, differentiation, development and the maintenance of adult epithelia in vertebrate animals. In retinoid-dependent tissues (including the retina), retinal-oxidizing ALDHs have been shown to display differential expression patterns during organogenesis in rodents, reflecting that RA signaling is indeed important for embryogenesis. The *in vivo* function of ALDH1A1 in RA synthesis is proven by the fact that after retinol treatment, while *Aldh1a1*$^{-/-}$mice are viable and possess normal morphology of the retina, the livers of *Aldh1a1*$^{-/-}$ mice have reduced RA synthesis and increased serum retinal levels. Surprisingly, it appeared that *Aldh1a1*$^{-/-}$ mice are protected against both diet-induced obesity and insulin resistance and this demonstrates that retinal might regulate the metabolic response to high-fat diets transcriptionally, and that the *ALDH1A1* could be a candidate gene for therapeutic targeting. Supression of *ALDH1A1* in cultured hepatocytes reduces both the omega-oxidation of free fatty acids and the production of reactive oxygen species (ROS). Liver ALDH1A1 levels were shown to be decreased in *RXRα*$^{-/-}$ mice, which suggests that RA binding is an activating factor in *ALDH1A1* gene expression. The androgen receptor might also be included in modulation of ALDH1A1, which is recognized to be an androgen binding protein. RA is required for testicular development and *ALDH1A1* is absent in genital tissues of humans with androgen receptor-negative testicular feminization. ALDH1A1 is significantly expressed in dopaminergic neurons that are known to require RA for their differentiation and development in the human brain. In these neurons, *ALDH1A1* is under the control of Pitx3, a homeodomain transcription factor that, possibly through *ALDH1A1* upregulation, regulates the particularization and maintenance of

disassociated populations of dopaminergic neurons. Decreased levels of ALDH1A1 takes place in dopaminergic neurons of the substantia nigra of patients with Parkinson's disease (PD), as well as the ventral tegmental area in schizophrenic patients. In the central nervous system (CNS), monoamine oxidase (MAO) metabolizes dopamine to aldehyde, as it's metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL), which growing evidence suggests might be neurotoxic, and it may lead to cell death in relation to neurological pathologies when accumulated. In maintaining low intraneuronal levels of DOPAL, ALDH1A1 may undertake a critical role by catalyzing its metabolism to 3,4-dihydroxyphenylacetic acid (DOPAC). Being one of 139 genes that are differentially expressed in primary human HSCs, and through the production of RA, ALDH1A1 has been shown to promote their differentiation.

These data suggest that for the therapeutic amplification of HSCs, ALDH1A1 inhibition could potentially be used (Marchitti, *et al.*, 2008, Moore, *et al.*, 2009).

3.1.2. ALDH1A2

ALDH1A2 is a cytosolic homotetramer expressed in several embryonic and adult tissues such as brain, kidney, intestine, testis, liver, retina, lung. As ALDH1A1, ALDH1A2 also catalyzes the reaction in which both all-*trans*-retinal and 9-*cis*-retinal oxidize to RA. However, when compared with other ALDH isozymes, ALDH1A2 appears to acquire the highest specificity (V_{max}/K_m = 49 nmol·min^{-1}·mg^{-1}·μM^{-1}) for all-*trans*-retinal. This characteristic may be because of an uncommon discreate loop in its active site that binds all-*trans*-retinal in a unique manner.

Taking action in several developmental processes, ALDH1A2 might be a key regulator of RA synthesis in developing tissues. Due to defects in early heart morphogenesis, *Aldh1α2*$^{-/-}$ mice die in early embryonic stages in which they seem to lack axial rotation, incomplete neural tube closure, reduction of the trunk region and many of the properties of human DiGeorge/velocardiofacial syndrome, a disorder characterized by cleft palate, heart abnormalities and learning disabilities. During early vascular development, aberrations in endothelial cell cycle progression have also been determined in *Aldh1α2*$^{-/-}$ embryos. *Aldh1a2* has been determined as a key regulator in the development of many tissues including kidney, retina, lung, forebrain, pancreas, and spinal cord by miscellanous animal models (Marchitti, *et al.*, 2008, El Kares, *et al.*, 2010).

3.1.3. ALDH1A3

ALDH1A3 is a cytosolic homodimer that participates in RA synthesis, oxidizes both all-*trans*-retinal and 9-*cis*-retinal (K_m 0.2 μM for all-*trans*-retinal) to RA, and has an important role in embryonic development; including brain, retina, skeletal muscle, tooth buds, intestine, kidney, prostate, lung, liver and pancreas, it is expressed in various late-stage embryonic and adult rodent tissues. In humans, ALDH1A3 expression has been noted in stomach, salivary gland, breast, kidney and fetal nasal mucosa. *Aldh1a3*$^{-/-}$ mouse embryos die as a result of defects in nasal development.

It's been shown that ALDH1A3 takes part in the development of the eye, nucleus accumbens and olfactory bulbs, the forebrain, hair follicles and the cerebral cortex.

ALDH1A3 deficiency has been shown to play a critical role in cancer by a number of studies. For instance, in human breast cancer MCF-7 cells, ALDH1A3 expression is downregulated, whereas in cultured human colon cancer cells, *ALDH1A3* is one of two genes that are upregulated by induction of wild type *p*53. In mammary tumor-susceptible BALB/cJ mice that are heterozygous for *p*53, *Aldh1a3* is one of five candidate genes located within a region determined for its linkage to mammary tumorigenesis. In mice resistant to induced mammary tumors, (C57BL/6J), *Aldh1a3* is one of the two upregulated genes. *ALDH1A3* is silenced by methylation in gastric cancer cells, whereas in glioblastoma cells, it is triggered by the antitumor agent IL-13 cytotoxin (Marchitti, *et al.*, 2008).

3.1.4. ALDH2

ALDH2 is a tetrameric enzyme expressed profusely in lungs and liver; it is also present in organs that obligate high mitochondrial capacity for oxidative ATP generation including heart and brain. Apart from that, ALDH2 is also important in the aldehydic substrate oxidation such as 4-HNE, acrolein, and short-chain, aromatic or polycyclic carbons. To add to its dehydrogenase activity, depending on the substrates, ALDH2 can function as an esterase and reductase. More recent attention has also been focused on ALDH2 in regards to its function in the biotransformation of nitroglycerin, reducing it to 1,2-glyceryl dinitrate for the production of nitric oxide, which is a critical vasodilator (Chen, *et al.*, 2010).

3.1.5. ALDH7A1

ALDH7A1 is a homotetramer that's expressed in a large number of tissues; in rat heart, liver and kidney, increased levels of ALDH7A1 are noted, whereas in black seabream fish (sbALDH7A1), ALDH7A1 is significantly formed in the liver and the kidney, excluding the heart. In human fetal tissues, ALDH7A1 has been encountered at elevated levels in the cochlea, eye, ovary, heart and kidney. In contrast, balanced levels are detected in the liver, spleen, muscle, lung and brain.

Human ALDH7A1's primary role happens in the pipecolic acid pathway of lysine catabolism, in which it catalyzes the oxidation reaction of alpha-aminoadipic semialdehyde (AASA) (K_m180 μM) to alpha-aminoadipate. *ALDH7A1* mutations form the molecular basis for pyridoxine-dependent epilepsy (PDE), an autosomal recessive disorder characterized by the aggression of tenacious seizures during infancy and early childhood and are avoidable by daily use of high-dose pyridoxine (Vitamin B_6) supplementation.

Remarkably, ADH7A1 expression in the cochlea of the ear, the region dependent on the healthy upkeep of internal hydrostatic pressure, clarifies that mammalian ALDH7A1 might have an accomplishable function in osmotic regulation and in hearing disorders. However, no connection has been revealed yet, including patients with the inner-ear disorder Ménière's disease, which effects hearing and balance.

ALDH7A1 is notably and differentially expressed within the first and second meiotic stages of porcine oocyte development. Screening of the promoter region *sbALDH7A1* has discovered *cis*-elements linked with cell cycle regulation (Marchitti, *et al.*, 2008).

4. ALDH in cancer and cancer stem cells

4.1. Adenocarcinoma

Adenocarcinoma is an epithelium cancer that is generated from glandular tissue. Epithelial tissue includes, but is not limited to, the surface layer of skin, glands and a variety of other tissues that line the cavities and organs of the body. Epithelium can be derived from the three germ layers ectoderm, mesoderm and endoderm during embryologic period. Adenocarcinoma classification depends on not only being a part of the gland, but also depends on having the same secretory characteristics. But, this form of carcinoma can occur in some higher mammals, including humans (Fauquier, *et al.*, 2003).

Adenocarcinomas can arise in many tissues of the body due to the ubiquitous nature of glands within the body. While each gland may not be secreting the same substance, as long as there is an exocrine function to the cell, it is considered glandular and its malignant form is therefore named adenocarcinoma. Endocrine gland tumors, such as a VIPoma, an insulinoma, a pheochromocytoma, etc. are typically not referred to as adenocarcinomas, but rather, are often called neuroendocrine tumors. If the glandular tissue is abnormal, but benign, it is called an adenoma. Benign adenomas typically do not invade other tissues and rarely metastasize, whereas malignant adenocarcinomas do both. Colon, urogenital (cervical (Tewari, *et al.*, 2002), prostate, urachus and vagina), breast (Buchholz, 2009), esophagus, pancreas, stomach and throat are several examples of adenocarcinoma (Subramanian & Govindan, 2007).

It is reported that ALDH expression marks pancreatic cancer stem cells. Also, they have mentioned that the enhanced clonogenic growth and migratory properties of ALDH-positive pancreatic cancer cells suggest a key role in the development of metastatic disease that negatively affects the overall survival of patients with pancreatic adenocarcinoma (Rasheed, *et al.*, 2010).

4.2. Breast cancer

From high-grade, absence of hormone receptor expression to positive HER2 status and the basal-like molecular subtype, the expression of ALDH1 is in direct relation with undesired tumor characteristics in breast cancer (Mieog, *et al.*, 2012).

Breast cancer cells with stem-cell-like properties are suggested to be responsible for metastatic spread. Aldehyde dehydrogenase 1 (ALDH1) and cluster of differentiation 44 (CD44) in addition to RhoC GTPase are among the stem cell markers that are expressed by these cells (Chaterjee & van Golen, 2011).

Breast CSCs were initially isolated, established on cell surface marker with CD24/lowCD44 expression. More currently, ''functional'' markers depending on stem cell properties are investigated for their plausible applications in the breast CSCs isolation. By this method, applying the aldefluor assay (Stemcell Technologies), originally designed to isolate viable HSCs and is an enzyme-based assay that recognizes ALDH activity, Ginestier et al. isolated breast CSCs. The assay is thought to precisely recognize ALDH isoform ALDH1A1 activity degree. Besides its application as a prognostic and CSC marker, ALDH activity that is primarily carried out by ALDH1A3 might be functional in breast cancer progression.

Expression of genes and tumor sphere formation in self-renewal and differentiation could be changed by adding chemical RA signaling inducers or inhibitors in breast cancer cell lines (Marcato, *et al.*, 2011).

ALDH1 could work as a marker of breast CSCs better than CD44+/CD24-. Though we could not maintain a conclusion that ALDH1 expression was significantly related with any conventional clinicopathologic attributes, nevertheless, there is a compelling relation between ALDH1-positive breast tumors and resistance to neoadjuvant chemotherapy, because of the pCR rates being obtained, which are lower in ALDH1-positive tumors (9.5%) than ALDH1-negative tumors (32.2%). Moreover, after neoadjuvant chemotherapy, a considerable increase in the proportion of ALDH1-positive tumor cells was observed. These results are an indication of ALDH1-positive tumor cells playing an important role in resistance to chemotherapy. Because of tumor cells being more tumorigenic than CD44+/CD24-, tumor cells of breast CSCs are thought to be richer in ALDH1-positive tumor cells than in CD44+/CD24- tumor cells. As a matter of course, we have shown that ALDH1-positive, in contrast with CD44+/CD24-, is closely associated with colony formation in the collagen gel as well. The subset of ALDH1-positive and CD44+/CD24- tumor cells has been reported to contain the largest proportion of breast cancer stem cells (BCSCs); consequently, it is speculated to have the strongest resistantance to chemotherapy. However, in our current study, pCR rates in the ALDH1-positive and CD44+/CD24- high subset (20%, 2 of 10), are not the lowest among all the subsets consisting of the ALDH1-positive and CD44+/CD24- low subset (0%, 0 of 11), the ALDH1-negative and CD44+/CD24- high subset (34.1%, 15 of 44), and the ALDH1-negative and CD44+/CD24- low subset (30.2%, 13 of 43). Adding CD44/CD24 status to ALDH1 status does not seem to positively improve the prediction of response to chemotherapy. Together, these results direct us to assume that, at least for the prediction of resistance to chemotherapy, ALDH1-positive tumor cells serve as a better marker for BCSCs than CD44+/CD24- tumor cells. Because such tumors contain a higher proportion of CSCs, we suppose that ALDH1- positive tumors are resistant to chemotherapy. However, because ALDH1 has been shown to play an important role in the resistance to chemotherapy in hematopoietic cells, ALDH1-positive tumor cells might be involved in resistance to chemotherapy, regardless of whether they are CSCs or not. In addition to deeper illumination of ALDH1's function in chemotherapy resistance in breast cancers, obtaining a significantly specific marker for BCSCs is necessary to enlighten an authentic role of BCSCs' chemotherapy resistance.

ALDH1-positive, in contrast to CD44+/CD24-, was tremendously related to sequential paclitaxel- and epirubicin-based chemotherapy resistance, and the expression of ALDH1 increased after neoadjuvant chemotherapy, which stands for an indication of BCSCs, determined by ALDH1, indeed having played a significant role in chemotherapy resistance. This means that ALDH1-positive appears to be a better marker than CD44+/CD24- in identifying BCSCs, at least for the prediction of resistance to chemotherapy (Tanei, *et al.*, 2009).

4.3. Lung cancer

Each year, approximately 171,000 new cases of lung cancer are diagnosed, and 160,000 individuals do not survive from the disease in the United States. This high incidence and mortality makes lung cancer one of the most common cancers and the leading cause of cancer death in men. Lung cancer is still the leading cause of death from malignant diseases worldwide in spite of the advances in surgical treatment and multimodality treatments (Hibi, *et al.*, 1998).

Cancer stem cells have attributed resistance of a smaller fraction of cells in the tumor bulk against chemotherapeutics. The isolation of CSCs is important for these reasons and have been isolated using a variety of stem cell markers and phenotypes. CD133 has recently been reported to identify tumor-initiating cells in non-small cell lung cancer (NSCLC). ABCG2 is also a stem cell marker of a variety of tissues and transporter responsible for the multidrug-resistance phenotype. However, it was demonstrated that many cells in NSCLC and SCLC cell lines show tumorigenic potential, regardless of ABCG2 and CD133 expression. Recently, ALDH activity has been used for isolation of these kinds of cells. Normal SCs were shown to contain higher levels of ALDH activity than their more differentiated progeny. ALDH-positive cells of tumors have higher proliferation rates, migration and adhesion ability, and metastatic potential than ALDH-negative cells. This may occur because that RA product of ALDHs is thought to participate in cellular differentiation and stem cell self-protection (Serrano, *et al.*, 2011).

4.4. Ovarian cancer

Epithelial ovarian cancer is the sixth most common cancer in women worldwide and it is still the most lethal gynecologic malignancy (Iorio, *et al.*, 2007). Application of new technologies for detection of ovarian cancer could have an important effect on public health, but to achieve this goal, specific and sensitive molecular markers are essential (Petricoin, *et al.*, 2002). Aldehyde dehydrogenase-1A1 (ALDH1A1) has been a valid marker among several malignant and non-malignant tissues in spite of several stem cell markers to identify CSCs. ALDH plays a role in the biology of TICs as well as being a stem cell marker. Because ALDH1A1 is implicated in chemo resistance pathways, it is questioned that targeting ALDH1A1 can effect cells resistant to chemotherapy and represent a potential target for cancer stem-cell-directed therapy. In a study, ALDH1A1 was investigated in ovarian cancer cell lines and patient samples and examined whether targeting ALDH1A1 sensitizes cells to

chemotherapy in both *in vitro* and *in vivo* ovarian cancer models. They showed that ALDH1A1 expression and activity have increased chemo resistant ovarian cancer cell lines. Most importantly, down-regulation of ALDH1A1 expression has sensitized normally chemo resistant tumors to both docetaxel and cisplatin *in vitro* and in mouse models. Besides being a stem cell marker, ALDH1A1 is also a viable target for therapy and a mediator of the aggressive phenotype (Landen, *et al.*, 2010).

4.5. Pancreatic cancer

Pancreatic adenocarcinoma is a highly lethal disease, which is usually diagnosed in an advanced state, and for which there is little or no effective therapies (Li, *et al.*, 2007). Therefore, finding markers to detect a malignant cell transformation at an early stage is very important. Researches demonstrated that the pancreas possesses ALDH activity, and ALDH is also present in the pancreatic cancer cells. Different from other cancer tissues (such as ovarian and lung cancer), the activity of ALDH does not differ in pancreatic carcinoma tissue compared to normal pancreatic tissue. Additionally, serum levels of ALDH were not significantly elevated in patients with pancreatic cancer in comparison to healthy controls (Jelski, *et al.*, 2011).

4.6. Prostate cancer

The latest estimates of global cancer incidence show that prostate cancer has become the third most common cancer in men, with half a million new cases each year, constituting almost 10% of all cancers in men (Quinn & Babb, 2002). Identifying the origin of cells in prostate cancer and its distant metastases may be important for the improvement of more effective treatment strategies and preventive therapies. Measurement of ALDH activity provides great contribution to functional identification and characterization of normal SCs and their malignant counterparts. ALDH activity is important for drug resistance, cell proliferation, differentiation, and response to oxidative stress of prostate cancer like other important cancers.

ALDH enzyme activity is used for the isolation of "stem-like" cells based on a developmentally conserved stem/progenitor cell function. In a study, high ALDH activity was used to isolate human prostate cancer cells with significantly enhanced clonogenic and migratory properties both *in vitro* and *in vivo*. Similar to other cancer tissues, the percentage of ALDHhi cells in prostate cancer cell lines are also related to tumorigenicity and metastatic behavior.

Although high expression of ALDH7A1 is shown in prostate cancer cell lines, primary cultures, and in primary prostate cancer tissue and matched bone metastases, ALDH3A2 and ALDH18A1 are not observed high ALDH activity in human prostate cancer (van den Hoogen, *et al.*, 2010).

4.7. Brain cancer

Glioblastoma (GBM) is the most common primary brain tumor in adults with an approximately 15-month survival (Stupp, *et al.*, 2005). Although there are several studies to

improve the postoperative therapeutic applications within the last few years, there is not enough succes for this highly aggressive tumor. After resection, radiation, and chemotherapy regimens, relapses occur regularly. Thus, it is thought that this can be a clue to the presence of tumor stem cells (TSCs). This cellular subfraction within GBM causes continuous tumor growth and resistance to drugs and radiation (Rasper, *et al.*, 2010). TSCs are believed to nestle in the tumor, keeping it alive and growing, providing pluripotency, self-renewal, and resistance to chemo and radiation therapy(Reya, *et al.*, 2001). The first malignancies from which cells could be isolated and showed the potential to self-renew and to drive tumor formation and growth were leukemias (Bonnet & Dick, 1997). After that, a stem cell subfraction was described in brain tumors (Singh, *et al.*, 2003). This was the first study that identified and showed a population with stem cell properties in pediatric solid brain tumors. Those cells were identified by their ability to proliferate under serum-free cell culture conditions and by the expression of CD133 and nestin. CD133 has long remained the most important TSC marker in malignant glioma. On the other hand, ALDH1 is a cytoplasmatic stem cell marker in a variety of malignant tumors and catalyzes the oxidation of intracellular aldehydes including the transformation of retinol to RA. As mentioned above, RA is a modulator of cell proliferation and differentiation that possibly contributes to the maintenance of an undifferentiated stem cell phenotype. Jones et al. presented a method to isolate human cells via flow cytometry depending on the amount of cytosolic ALDH (Jones, *et al.*, 1995). Recently, Ginestier et al. found ALDH1 to be a stem cell marker in breast carcinoma associated with poor clinical outcomes (Ginestier, *et al.*, 2007). Since then, ALDH1 has been described as a marker of stemness in other solid malignancies including lung cancer (Jiang, *et al.*, 2009) and colorectal cancer (Huang, *et al.*, 2009).

Therefore, identification and isolation of these cells seem crucial for a better understanding of tumor behavior, origin, and therapy. Recently, ALDH1 has been described as a marker for the identification of non-neoplastic SCs and TSCs (Ginestier, *et al.*, 2007).

So far, cellular markers including CD133 have been used to identify TSCs in GBMs, but recently, CD133-negative GBMs are characterized to behave as brain TSCs (Beier, *et al.*, 2007).

Therefore, ALDH1 has also been described as a stem cell marker in various solid neoplasms including lung cancer (Jiang, *et al.*, 2009), breast carcinoma (Ginestier, *et al.*, 2007), and colorectal cancer (Huang, *et al.*, 2009) and GBM (Rasper, *et al.*, 2010).

4.8. Colon cancer

Most colon cancers are adenocarcinomas that release mucus and other cellular secretions. In the United States in 2012, estimated new cases and deaths from colon and rectal cancer are reported as: 103,170 colon cancers and 51,690 deaths (Levin, *et al.*, 2008). Studies showed that ALDH1B1 and ALDH1A1 are differentially expressed in normal human tissues, but ALDH1B1 is expressed at higher levels than ALDH1A1 in human epithelial cancers. ALDH1B1 was abundantly expressed in adenocarcinomas originating from the tissue and particularly in colonic adenocarcinoma (Chen, *et al.*, 2011). Thus it can be deduced that ALDH1B1 may be a marker for colon cancer diagnosis.

5. Aldefluor activity in stem cells and cancer stem cells

$ALDH^{br}$ (ALDH-bright) cells can be detected with ALDEFLUOR reagent by using flow cytometry or fluorescent microscopy. These $ALDH^{br}$ cell populations are isolated from adult tissues by flow sorting.

ALDH activity was shown in human and mouse bone marrow hematopoietic progenitor cells (HPCs) by Jones et al. for the first time (Jones, *et al.*, 1995). ALDH was assayed by using a new substrate with low light scatter properties with flow cytometry. Now this method was improved and known as Aldefluor assay. Aldefluor assay can be used for to measure ALDH activity of adult tissue cells, primary cancer cells and cultured cells. Aldefluor assay is based on the conversion of fluorescent non-toxic substrate for ALDH substrate to the fluorescent reaction product. Non-toxic substrate for ALDH freely diffuses into intact and viable cells. The BODIPY aminoacetaldehyde is converted to the fluorescent product BODIPY aminoacetate by ALDH activity (Figure 1). In this assay, a specific inhibitor of this reaction (diethylaminobenzaldehyde-DEAB) is used to control for background fluorescence. Aldehyde dehydrogenase plays a role as a cancer stem cell marker comes down to the specific isoform.

Stem and progenitor cells are identified as cells with low side scatter and high expression of ALDH. DEAB allows to distinguish between ALDH-bright cells and cells with low ALDH activity. Generally, 10^5-10^6 cells are suspended in Aldefluor assay buffer containing BODIPY aminoacetaldehyde with/without DEAB. Aldefluor was excited at 488 nm and fluorescence emission was detected at 530/30 (van den Hoogen, *et al.*, 2010). This assay provides a successful isolation of viable HSCs and more recently ALDH positive CSCs. However, aldefluor assay detects the ALDH activity of several ALDH isoforms expressed in the cells. ALDH1A1 is not the only isoform responsible from aldeflour activity. In some studies, it was demonstrated that ALDH1A1-deficient hematopoietic cells showed aldefluor activity owing to ALDH2, ALDH3A1 and ALDH9A1 isoforms (Marcato, *et al.*, 2011).

6. ALDH bright ($ALDH^{br}$) cell

Intracellular ALDH enzymes are responsible for oxidizing aldehydes to carboxylic acids in the cell. $ALDH^{br}$ cells from different tissues express high ALDH activity and have progenitor cell activity (Gentry, *et al.*, 2007). Firstly, HSC were defined as $SSC^{lo}ALDH^{br}$ – reflecting their low orthogonal light scattering and bright fluorescence intensity by using flow cytometry (Lioznov, *et al.*, 2005). After that, high levels of the enzyme ALDH ($ALDH^{br}$) have proven to be a novel marker for the identification and isolation of SCs (Mitchell, *et al.*, 2006). In the same time angiogenic activity of $ALDH^{br}$ cells were discovered and these cells were used for regenerative medicine with preclinical models and have been used safely to treat patients in early clinical trials (White, *et al.*, 2011).

$ALDH^{br}$ cells were found in various cancer tissues including breast, liver, colon, and acute myelogenous leukemia and related with cancer chemo resistance. Human and murine HSCs and neural stem and progenitor cells have increased ALDH activity compared to non-stem-cells (Siclari & Qin, 2010).

Therefore, recently the importance of ALDH activity in normal and malignant stem cell functions, and the potential diagnostic and therapeutic implications gain importance (Moreb, 2008).

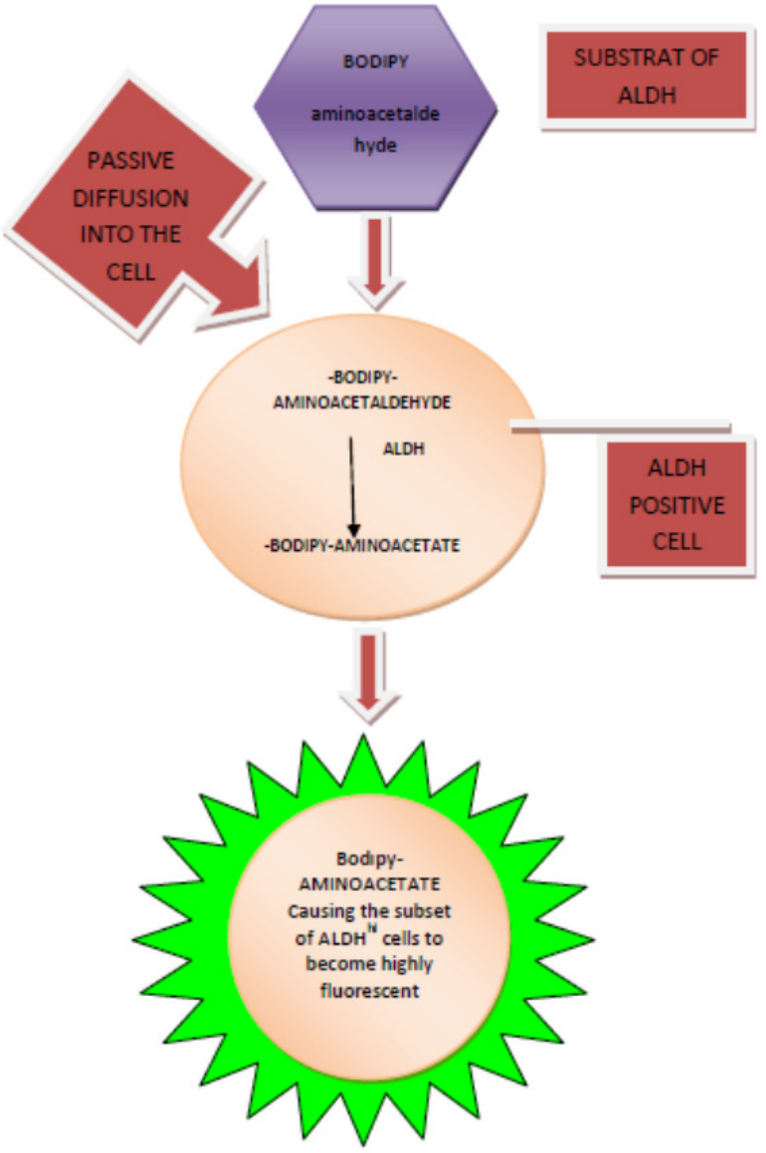

Figure 1. The Aldefluor® Assay. Firstly, ALDH positive cell will uptake BODIPY-aminoacetaldehyde by passive diffusion and then convert BODIPY-aminoacetaldehyde into BODIPY-aminoacetate. Then BAA is retained inside cells, causing the subset of ALDHhi cells to become highly fluorescent (Marcato, *et al.*, 2011).

7. Aldehyde dehydrogenase in regenerative medicine

Until today, studies showed that BM $ALDH^{br}$ populations may be useful in several cell therapy applications (Gentry, *et al.*, 2007). According to this information, $ALDH^{br}$ population

may play an important role in regenerative medicine owing to RAs ALDH product (Balber, 2011). Retinoic acids could influence tissue repair by binding to transcription factors and regulating developmental programs, especially ALDH1A1 and ALDH3A1 of enzyme isoforms that produce RAs from oxidize retinaldehyde (Moreb, 2008). Therefore, ALDH1a1 and ALDH1A3 may influence cell activity and proliferation by controlling intracellular retinoid concentrations and play important roles in stem cell biology (Balber, 2011).

The studies about value of $ALDH^{br}$ cells in regenerative medicine were conducted by different researchers. The regenerative potential of $ALDH^{br}$ cells obtained from different tissues were investigated in various disease models such as ischemic tissue damage hind limb model, brain damage and pancreatitis (Balber, 2011).

In the beginning of studies, $ALDH^{br}$ cells were obtained from bone marrow and umbilical cord blood and normal peripheral blood (Sondergaard, *et al.*, 2010). Multipotent mesenchymal progenitors and endothelial progenitor cells are concentrated in human $ALDH^{br}$ populations. Because of potential progenitor and paracrine activities of $ALDH^{br}$ cells, these cells especially obtained from bone marrow are important for tissue repair.

Manipulation of the graft to selectively concentrate or expand hematopoietic and/or neural stem cells prior to transplant may be a potential strategy in the future. UCBT using ALDH bright cells from the CB units have shown faster and higher engraftment in preliminary study and is being explored further (Prasad & Kurtzberg, 2010). One of these studies showed that human cord blood progenitors with high ALDH activity improve vascular density in a model of acute myocardial infarction. In this study, $ALDH^{br}$ cells were homed to the infracted anterior surface of the heart, while ALDH-low cells were in the spleen after intravenously administration.

Another study with animal model of hindlimb ischemia demonstrated that the isolated $ALDH^{br}$ cells effectively restored blood flow to ischemic areas by mediation of local formation of new blood vessels with largeer diameter and increasing capillary density even if there was no improvement in cardiac functions (Keller, 2009).

The reason for the restoration of tissue perfusion by $ALDH^{br}$ cells were attempted to be explained with angiogenic properties of these cell groups. Angiogenic factors secreted by transplanted $ALDH^{br}$ cells stimulate formation of new blood vessels at sites of ischemic injury (human cord blood progenitors with high ALDH activity improve vascular density in a model of acute myocardial infarction). Paracrine mechanisms of $ALDH^{br}$ cells can protect endothelial cells from ischemic damage and respond to ischemic tissue damage (Balber, 2011, White, *et al.*, 2011).

Another exciting finding is that $ALDH^{br}$ cells improve formation of new vessels and increase capillary density, while $ALDH^{br}$ cells together with ALDH-low cells did not restore tissue perfusion at all. It is suggested that ALDH-low cells can inhibit the homing and/or angiogenic activity of $ALDH^{br}$ cells. This situation showed the importance of isolating $ALDH^{br}$ cells from bone marrow tissue for therapeutic uses (Balber, 2011). As a result, $ALDH^{br}$ cells may be promising for patients with ischemic heart failure and critical limb ischemia (Keller, 2009).

TISSUE	OBTAINED FROM	BENEFITS
Neural Tissue	Rat embryonic neural tube Fetal mouse brain Subventricular and subcortical zones of adult mouse brain	Ability to form neurospheres and retained multipotency Transplantation significantly ameliorated disease progression and extended life, but did not rescue the animals.
Skeletal Muscle	Biopsies or primary explants of human skeletal muscle	Strong myogenic potential on IM transplantation
Mammary Epithelium	Mammary epithelium	Myoepithelial, luminal epithelial and mixed colonies, and ducts, when transplanted into mammary fat pads.
Pancreatic Cells	Central acinar/terminal duct cells from peripheral acinar duct units of adult mice	Contributed to both exocrine and endocrine lineages in the developing pancreas
Prostate Epithelium	-	Express basal epithelial and characteristic prostate progenitor cell markers
Corneal Limbic Cells	Cadaveric human limbic tissue	Protects the cornea from oxidative damage

Table 1. Different tissue repair models including human ALDH^{br} cells (Balber, 2011).

8. Conclusion

Since ALDH enzyme has been proven to possess a vital role in somatic cells and their deficiency cause various diseases, research has focused on the presence and functions of the enzyme in SCs. It was demonstrated that ALDH is an important marker for identification of SCs and has several functions in these cells just as they possess in somatic cells.

Exploring some of the isoforms of ALDH for use as a marker of CSCs improved the importance of ALDH. Thus, there are several methods to detect ALDHs and their levels (Marcato, *et al.*, 2011). After the discovery of ALDH activity in human and mouse bone marrow hematopoietic progenitor cells (HPCs) by Jones et al. (Jones, *et al.*, 1995), the properties and locations of ALDH-positive cells have started to be investigated.

Recently, ALDH^{br} cells were found in cancer tissues including breast, liver, colon, and acute myelogenous leukemia. It was demonstrated that proliferation rates, migration and adhesion ability, and metastatic potential of ALDHbr CSCs were more than ALDH low cells and ALDH^{br} cells related with cancer chemo resistance. ALDHbr cells became one of new therapeutic target against cancer and anti-cancer studies based on targeting ALDHbr cells have started recently (Serrano, *et al.*, 2011). It is expected that the anti-cancer studies with this perspective may intensively continue.

On the other hand, studies showed that BM ALDH^{br} populations may be useful in several cell therapy applications (Gentry, *et al.*, 2007). It is suggested that ALDH^{br} population may play an important role in regenerative medicine owing to Ras, which are one of the ALDH products. Paracrine effects of products of ALDH activity may influence tissue repair by binding to transcription factors and regulating developmental programs (Balber, 2011).

Therefore, regenerative potential of ALDHbr SCs were investigated in various disease models such as ischemic tissue damage hind limb model, brain damage and pancreatitis (Balber, 2011).

Studies on ALDH^{br} cells provide restoration of tissue perfusion and stimulation of formation of new blood vessels in ischemic tissue damage (Keller, 2009). These promising findings showed that ALDH^{br} cells may gain importance in different areas; however, there are still many things to investigate about potential properties of ALDH^{br} cells for use in regenerative medicine. Thus, ALDH have many roles such as a marker of many disease and cell lines for detection of them also can using for therapy and have potential for use in regenerative medicine.

However, there are few studies about ALDH as a marker of SCs and potential usage in regenerative medicine. Therefore, we suggested that studies should focus on this and this review aims to consider the roles of ALDH in SCs and their potential use in regenerative medicine. We believe that constructing a review including current studies related to this subject will guide future studies.

Author details

Adil M. Allahverdiyev, Malahat Bagirova, Olga Nehir Oztel,
Serkan Yaman, Emrah Sefik Abamor, Rabia Cakir Koc,
Sezen Canim Ates and Serap Yesilkir Baydar
Department of Bioengineering, Yildiz Technical University, Istanbul, Turkey

Serhat Elcicek
Department of Bioengineering, Yildiz Technical University, Istanbul, Turkey
Department of Bioengineering, Firat University, Elazig, Turkey

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