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Meta-analysis

A meta-analysis evaluating the role of calcium channel alpha-2 delta-1 subunit in carcinogenesis

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ABSTRACT

There is hardly found any study accumulating all the experiments reported with the expression of alpha-2 delta-1 (α2δ-1) in cancer cells. This meta-analysis aimed to advance our knowledge about the role of calcium channel alpha2 delta-1 subunit in carcinogenesis in the present time. PubMed searches for peer-reviewed articles were conducted using the keywords "α2δ-1 protein in oncogenesis", "α2δ-1 protein expression in cancer cells", and "α2δ-1 protein as cancer cell marker". The databases were developed in accordance with PRISMA guidelines. Seventeen studies out of 80 citations met the inclusion criteria pertaining to α2δ-1 expression in different cancer cells. The cancer patterns were hepatocellular carcinoma in 41%, non-small cell lung carcinoma in 12% and laryngeal squamous cell carcinoma in 12%. The remaining studies included small-cell lung cancer (6%), gastric cancer (6%), pancreatic cancer (6%), hypopharyngeal squamous cell carcinoma (6%), breast cancer (6%) and glioblastoma multiforme (6%). α2δ-1$^+$ cells had a higher sphere-forming and tumorigenic efficiency in 76.5% of experiments. 58.8% experiments explored mechanistically in self-renewal efficiency and tumorigenesis of α2δ-1$^+$ cancer cells. The cancer cells expressing α2δ-1 have the potential to serve as cell surface markers for tumour-initiating cells and cancer stem cells. These intriguing findings open up a promising avenue for future research, focusing on the targeting of α2δ-1 as a potential therapeutic strategy for cancer treatment.

Keywords: Meta-analysis, Alpha-2 delta-1 protein, Carcinogenesis

INTRODUCTION

19.3 million people are predicted to get cancer each year, leading to an estimated 10.0 million cancer deaths, according to the International Agency for Research on Cancer (IARC). In reality, 1 in 5 people worldwide will develop cancer at some point in their lives, and 1 in 8 men and 1 in 11 women will pass away from the disease. From the expected 19.3 million cases in 2020, the overall incidence of cancer is projected to grow by 47% (28.4 million new cases) by 2040.[1]

It is well known that the normal cells transit into the cancer cells with the emergence of a multi-stage transforming process. It incorporates rise of two particular subpopulations of self-renewing cells such as tumour-initiating cells (TICs) and cancer stem cells (CSCs). TICs has the capacity of tumour initiation, whereas CSCs has the capacity of tumour maintenance. It is worthy to mention here that the terms TICs and CSCs are not interchangeable, though TICs bear stem cell-like properties.[2-4]

The patterns of cancer progression are associated with the emergence of "cancer hallmarks" such as deregulated cell proliferation, resistance against cell death, angiogenesis, invasion and metastasis.[5,6] The cancer metastasis contributes to the burden of cancer cases and most of the cancer related death.[7] A current threat to the world is the advent of multiple drug resistance to anticancer therapy. The emergence of chemo-resistance is responsible for up to 90% of the cancer related deaths.[8-12]

Ions and ion channels play a critical role in cell functioning and excitation each in prokaryotes and eukaryotes. Due to its distinct characteristics, calcium stands out as an exceptional ion in biological systems. Calcium possesses unique qualities, as it has the ability to generate electrical signals and membrane potentials. Furthermore, it plays a crucial role as an indispensable component within cell signalling molecules. Therefore, calcium channels play an even more crucial role in cellular functions due to their ability to facilitate a diverse array of cellular reactions. Recent findings indicate that abnormal calcium signalling is strongly associated with the development and proliferation of cancer cells. This intricate connection has been observed specifically in relation to the fundamental characteristics of cancer, also referred to as the hallmarks of cancer. These characteristics comprise uncontrolled expansion, invasion of nearby tissues, and movement to distant places. Accumulating evidences suggest that various membrane receptors including voltage gated ion channels are deregulated and expression differently in cancer cell population.[13-19]

The α2δ subunits, known as auxiliary subunits of voltage-gated calcium channels, are now gaining attention as important regulators of many critical processes involved in synaptic function regulation. These occurrences involve the complex procedures of channel trafficking and localization, which are crucial for the correct operation and dispersal of calcium channels in the synapse.[20,21]

Moreover, the α2δ subunits have been discovered to have a crucial involvement in the initial formation of synaptic structures.[22] This further highlights their importance in the overall coordination of synaptic function. Moreover, few evidence indicates that cancer cells expressing alpha-2 delta-1 protein (α2δ-1), unlike conventional cell surface markers that encourage the growth and persistence of tumors, may function as possible markers for TICs and CSCs by serving as an auxiliary component of voltage-dependent calcium channels.[23,24]

Truly speaking, there is hardly found any study accumulating all the experiments reported with the expression of α2δ-1 in cancer cells. The present study is designed to advance our knowledge about the role of α2δ-1 in oncogenesis and to explore the mechanisms through which these proteins exert their regulatory influence on oncogenesis. In doing so, we hope to unravel the intricate web of molecular interactions that underlie the initiation and perpetuation of cancer.

METHODS

The present study was designed as a meta-analysis to identify the role of α2δ-1 in oncogenesis and to describe the expression of α2δ-1 as marker in relationship to cancer. The study was done in the department of molecular biology and bioinformatics of Tripura University (a central University), Tripura, India.

Search strategy

A systematic search of the PubMed database was made in order to identify all of the articles containing the terms "α2δ-1 protein expression in cancer cells", "alpha2 delta-1 protein as cancer cell marker", "α2δ-1 protein in oncogenesis", and "α2δ-1 in cancer". Language was restricted to English. The search was conducted on 04 January 2024. A thorough manual review of the experimental studies was performed using cross-references from identified original articles.

Selection criteria

Studies meeting the following criteria were considered for inclusion: α2δ-1 expression in cancer cells in the experimental setting, α2δ-1 as cancer cell marker, α2δ-1 in oncogenesis, and α2δ-1 in cancer. We included only experimental studies retrieved in full text. A two-stage study selection process was used: all titles and abstracts were initially screened for potential relevance, with full texts of the potentially relevant references being screened subsequently. The exclusion criteria were experiments those do not neatly fit into the inclusion criteria and studies that publish in other than English language.

Data extraction

The author independently performed the literature search and screened all study titles and abstracts. From the eligible articles, full-text versions were retrieved and reviewed. Data were extracted and checked.

The authors collected information to develop the study about sphere formation assay, tumorigenicity assay, and mechanistic exploration of α2δ-1^{+} cancer cells. Additionally, the authors explored the details of cell lines, clinical samples, xenograft and clinical cases. The authors also collected the immunofluorescence, immune-histochemistry, Western blot, and the serum level of α2δ-1 subunit in cancer case records if sphere formation assay, and tumorigenicity assay are not done.

The databases were developed in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.[25]

Descriptive statistics were used to demonstrate baseline characteristics and categorical variables of the experiments expressed as numbers and percentages.

RESULTS

Database searches conducted up until the date of 01 January 2024 have successfully identified a total of 105 citations, all of which possess publication dates falling within the range of 01 January 2000 to 01 January 2024. It is important to note that after removing 25 duplicate citations, a remaining sum of 80 reports were left for the purpose of screening. Upon completing the screening

process, it was determined that 18 articles remained and were subsequently assessed for eligibility. However, there was one article that could not be retrieved, resulting in a final count of 17 articles available for eligibility assessment. By adhering to the guidelines outlined by PRISMA, it was determined that all 17 of these articles satisfied the inclusion criteria (Figure 1).

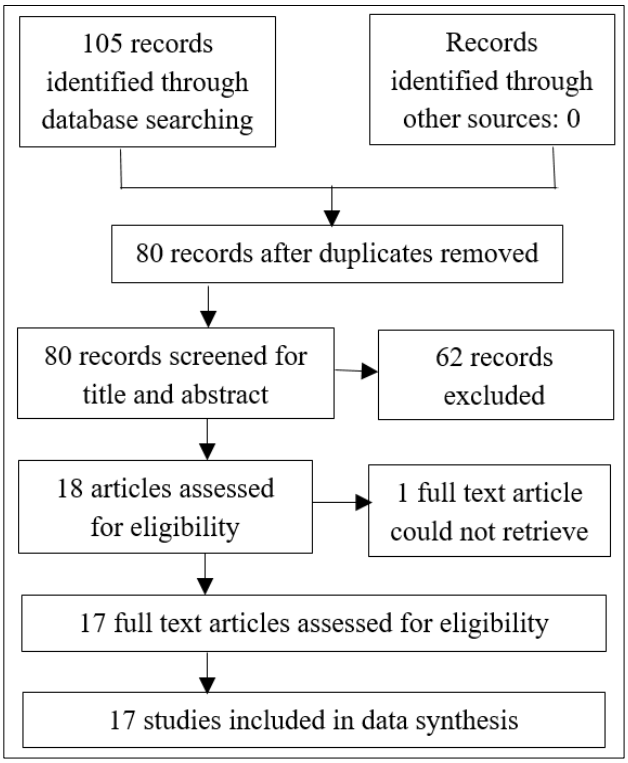

Figure 1: Flow diagram of the study selection process. Diagrammatic representation of study selection flow conducted according to the PRISMA statement.

In terms of the methodology employed, the experiments conducted were aggregated in a quantitative manner rather than being subjected to qualitative analysis. These 17 articles, upon analysis, provided a comprehensive overview of a total of 9 distinct cancer patterns (Figure 2). Among the identified cancer patterns, hepatocellular carcinoma (HCC) was reported in seven studies, accounting for 41% of the cases examined. Non-small cell lung carcinoma (NSCLC) was found in two studies, corresponding to 12% of the cases, while laryngeal squamous cell carcinoma (LSCC) was identified in two studies as well, representing an additional 12% of the cases. The remaining studies encompassed a variety of other cancer types, including small-cell lung cancer (SCLC), gastric cancer (GC), pancreatic cancer (PC), hypopharyngeal squamous cell carcinoma (HSCC), breast cancer (BC), and glioblastoma multiforme (GBM), each accounting for 6% of the cases respectively.

It was duly noted, through careful observation and analysis, that the chronology of published experiments, in their entirety, exhibited a distinct lack of steadfastness and consistency, if one were to categorically classify them. This particular finding is visually represented in Figure 3, where one can discern the fluctuations and variations in the temporal ordering of these experiments. Specifically, it is imperative to highlight that the initial instance in which the alpha2 delta-1 protein was identified as a cancer cell marker dates back to the year 2013, while the most recent and final experiment pertaining to this marker was conducted and documented in the year 2023. It is crucial to emphasize that these experiments were conducted and documented within the predetermined and stipulated period of time, as dictated by the parameters of the database searching process.

Within the diverse array of experiments, various types of samples were utilized, each serving a unique purpose in contributing to the overall understanding of the subject matter (Table 1). Specifically, cell lines were utilized in a vast majority of the experiments, precisely 15 out of the 17 conducted, constituting a substantial 88.2% of the study population. Additionally, clinical samples were employed in a total of 9 experiments, accounting for approximately 52.9% of the entire study population. Lastly, xenograft experiments, involving the transplantation of cells or tissues from one species into another, were conducted in a total of 8 experiments, accounting for 47.1% of the study population. It is worth noting that, within this comprehensive array of experiments, only one study, representing a mere 5.9% of the entire study population, was classified as a clinical case study.

Table 1: The basic characteristics of study sample.

Variables	Number (n=17)	Percentage (%)
Clinical samples	9	52.9
Cell lines	15	88.2
Xenograft	8	47.1
Clinical case study*	1	5.9

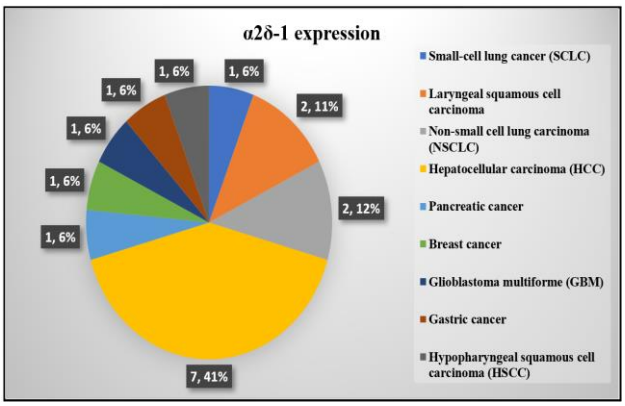

Figure 2: Distribution of 17 experiments demonstrated α2δ-1 expression in different cancers. The data levels represent the number and percentage of data records.

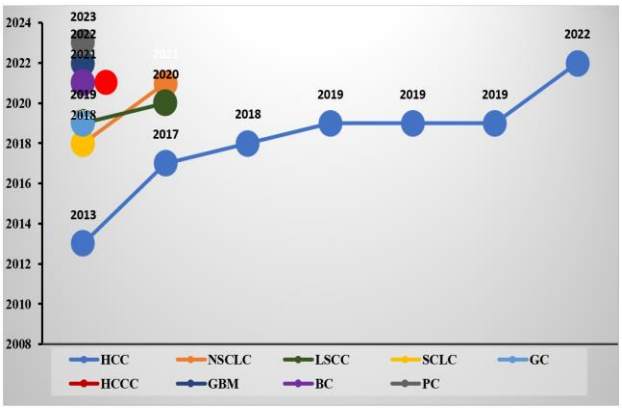

Figure 3: Year wise chronology of the data records of 9 different cancers: category wise chronology of all 17 experiments.

HCC: Hepatocellular carcinoma, NSCLC: non-small cell lung carcinoma, LSCC: laryngeal squamous cell carcinoma, SCLC: small-cell lung cancer, PC: pancreatic cancer, BC: breast cancer, GC: gastric cancer, HSCC: hypopharyngeal squamous cell carcinoma, GBM: glioblastoma multiforme

The sphere formation assay, which was conducted in a total of thirteen (76.5%) experiments, provided evidence of a higher efficiency in the formation of spheres by α2δ-1+ cells. Furthermore, the tumorigenicity assay, also performed in thirteen (76.5%) experiments, revealed a greater efficacy in inducing tumorigenicity by α2δ-1+ cells. In the subset of experiments where the sphere formation and tumorigenicity assays were not conducted, further investigations utilizing immunofluorescence and western blot techniques were carried out, leading to the discovery of an overexpression of α2δ-1 in tissues affected by LSCC. Additionally, through the utilization of luciferase assays and Western blot analyses, the over expression of α2δ-1 in GBM cells was demonstrated. A separate study presented findings indicating that the serum level of the α2δ-1 subunit could potentially serve as a novel diagnostic biomarker for HCC. Another study focused on the prognostic significance of α2δ-1 in tissues affected by HSCC (Table 2).

Ten experiments, accounting for 58.8% of the total, were conducted to meticulously investigate the mechanistic aspects pertaining to the pivotal role played by CXCL11, PBX3 (also known as pre-B-cell leukemia transcription factor 3), ISL1 (commonly referred to as Islet1), IP3R2-mediated Ca2+ oscillation, LOX (otherwise known as Lysyl oxidase), Erk, NOTCH3, DNA damage repair proteins, PKM2, and TLR-4 (Table 3). These experiments aimed to explore the intricate relationship between these factors and their influence on the self-renewal efficiency and tumorigenesis of α2δ-1+ cancer cells. Among the mechanistic exploration studies, HCC was reported in five studies, accounting for 50% of the experiments examined. NSCLC was found in two studies, corresponding to 20% of the experiments, while SCLC, PC, and GBM, each accounting for 10% of the experiments respectively (Figure 4).

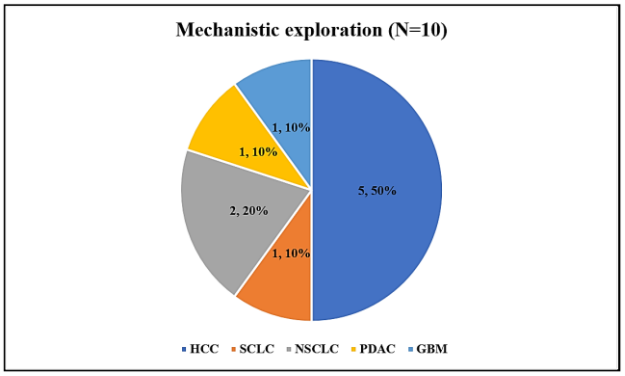

Figure 4: The percentage distribution of the experiments underwent mechanistic exploration based on different cancer patterns.

HCC: Hepatocellular carcinoma, NSCLC: non-small cell lung carcinoma, SCLC: small-cell lung cancer, PDAC: pancreatic duct adenocarcinoma, GBM: glioblastoma multiforme

Table 2: The basic investigations to explore the role of α2δ-1 in cancer.

Variables	Number of experiments (%)	Results
Sphere formation assay* (self-renewal property)	13 (76.47)	Higher sphere-forming efficiency of α2δ1+ cells
Tumorigenicity assay**	13 (76.47)	More tumorigenic of α2δ1+ cells
Immunofluorescence and western blot ***	1 (5.88)	α2δ1 was highly expressed in LSCC tissues compared with adjacent normal tissues ($p<0.05$)
Luciferase assay western blot ***	1 (5.88)	Higher expression of $\alpha2\delta1^{+}$ cells in GBM
IHC***	1 (5.88)	α2δ1 could serve as a new prognostic biomarker for HSCC
Serum level of α2δ1***	1 (5.88)	Serum level of α2δ1 subunit may be a novel diagnostic biomarker

*HCC [6], LSCLC [2], LSCC [1], SCLC [1], PC [1], BC [1] and GC [1]; ** HCC [6], LSCLC [2], LSCC [1], SCLC [1], PC [1], BC [1] and GC [1]; ***The experiments where sphere formation assay and tumorgenicity assay were not done, the authors considered these experiments respectively in LSCC [1], GBM [1], HSCC [1] and HCC [1]. The number within the parenthesis denotes the number of experiments. HCC: hepatocellular carcinoma, NSCLC: non-small cell lung carcinoma, LSCC: laryngeal squamous cell carcinoma, SCLC: small-cell lung cancer, PC: pancreatic cancer, BC: breast cancer, GC: gastric cancer, HSCC: hypopharyngeal squamous cell carcinoma, GBM: glioblastoma multiforme

Table 3: Mechanistic exploration based on different cancer patterns.

Cancer categories	Variables (mechanis-TIC explora-tion)	Result (self-renewal efficiency and tumorigenesis of α2δ1+ cells)
HCC (n=5)	CXCL11	The CXCL11 chemokine plays a crucial role in enhancing the self-renewal capacity and tumorigenic potential of liver tumor-initiating cells that express α2δ1. This effect is mediated through the activation of the CXCR3 receptor and subsequent activation of the ERK1/2 signaling pathway.[42]
	Pre-B-cell leukemia transcription factor 3 (PBX3)	PBX3, a protein that is indispensable for the acquisition and maintenance of TIC properties. Furthermore, PBX3 plays a crucial role in activating a transcriptional program that leads to the expression of genes essential for HCC TIC stemness, including CACNA2D1, EpCAM, SOX2, and NOTCH3. Additionally, the expression levels of CACNA2D1 and PBX3 mRNA can serve as prognostic indicators for poor outcomes in HCC patients. This uncovers a critical signaling pathway that regulates the transition of HCC TIC phenotypes.[43]
	Islet 1 (ISL1)	The phenomenon of overexpression of miR-31 in α2δ1 + hepatocellular carcinoma (HCC) tumor-initiating cells (TICs) leads to a significant and noteworthy suppression of the abilities of these cells to self-renew and form tumors. On the contrary, when the expression of miR-31 is intentionally reduced or knocked down in PLC/PRF/5 cells, a human HCC cell line, it is indeed possible to reprogram these cells into TICs that possess properties resembling those of stem cells. In a related context, it has been observed that miR-31 is capable of exerting a negative regulatory influence on the self-renewal capacity of α2δ1+ liver TICs through the process of silencing the ISL1 gene.[44]
	Ca^{2+} oscillation	Ca2+ oscillation is a distinctive characteristic of α2δ1+ CSC fractions from hepatocellular carcinoma cell lines controlled by the type 2 inositol 1,4,5-trisphosphate receptor (IP3R2).[45]
	Lysyl oxidase (LOX)	α2δ1 subunit-positive tumor-initiating cells of hepatocellular carcinoma have been observed to specifically secrete LOX, an enzyme that plays a crucial role in the crosslinking of collagen molecules. This process results in the formation of a rigid extracellular matrix, which in turn promotes the development of tumor-initiating cells that possess an inherent mechanical stiffness. It is important to note that this mechanical trait is substantial enough to facilitate the initiation and progression of tumor growth within the affected tissues.[46]
SCLC (n=1)	Extracellular signal-regulated kinase (Erk)	At the protein level, Erk activation and over-expression were observed in the α2δ1 positive H1048 cell line, highlighting its significance in cellular mechanisms.[47]
NSCLC (n=2)	NOTCH3	The impact of α2δ1 on TIC capacities is influenced by Notch signaling. α2δ1 has the ability to control the expression of NOTCH3. The upregulation of NOTCH3 is facilitated by α2δ1 through the Ca2+-Calcineurin/NFATc2 signaling pathway.[24]
	DNA damage repair proteins	Analysis of proteins involved in the repair of DNA damage has indicated that α2δ1 plays a role in enhancing the efficiency of this repair process. In particular, the monoclonal antibody targeting α2δ1 has been found to have a synergistic effect with radiation, effectively inhibiting the self-renewal of α2δ1-high cells and increasing the radiosensitivity of α2δ1-positive cells in colony formation assays. Furthermore, when combined with radiation, the α2δ1 antibody has been shown to suppress the growth of A549 xenografts in vivo.[48]
PC (n=1)	PKM2 (pyruvate kinase M2)	The role of α2δ1 in promoting the properties of pancreatic tumor-initiating cells (TICs) involves a series of phosphorylation events on PKM2, which are mediated by calcium/calmodulin-dependent protein kinase II delta (CaMKIIδ).[49]
GBM (n=1)	Toll-like receptor (TLR-4)	The expression of α2δ-1 is regulated by the activation of TLR-4 through the signaling pathway of NF-kB/Sp1.[50]

TICs: tumour-initiating cells, CSCs: cancer stem cells, α2δ-1: alpha2delta-1protein, PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses, HCC: Hepatocellular carcinoma, NSCLC: Non-small cell lung carcinoma, LSCC: Laryngeal squamous cell carcinoma, SCLC: Small-cell lung cancer, PC: Pancreatic cancer, BC: Breast cancer, GC: Gastric cancer, HSCC: Hypopharyngeal squamous cell carcinoma, GBM: Glioblastoma multiforme.

DISCUSSION

The conventional cell surface markers having CSC-like properties are CD133 (a 5-pass transmembrane glycoprotein), CD44 (a hyaluronic acid receptor), CD24 (a heat stable antigen), CD117 (a transmembrane protein with tyrosine kinase activity), CD87 (an urokinase-type plasminogen activator receptor) and aldehyde dehydrogenases (ALDH).[26-32] The most used stem cell marker among them for locating CSCs in different cancer cells is CD133.[33-38] The present meta-analysis suggests that the cancer cells positive for α2δ-1 is a potential TIC and CSC cell surface marker. This proves that α2δ-1-positive cancer cells, acting as an ancillary subunit of voltage-dependent calcium channels, may act as a potential TIC and CSC cell surface marker in contrast to the traditional cell surface marker which fosters tumour initiation and maintenance, respectively.

At the current moment, no biomarker has been established as an "ideal" cancer screening tool that can meet the diagnostic, prognostic, and predictive requirements simultaneously. Thus, the validation of new cancer biomarkers for efficient cancer diagnosis that is determination of clinical relevance and applicability is quite necessary and challenging. The notable advancement in the field of genomic and proteomic research has consequently resulted in a striking and remarkable surge in the ability to identify a wide array of potential genomic and proteomic cancer biomarkers. However, it is important to note that despite these significant strides made in the scientific domain, the translation of these particular findings into the realm of clinical practice has been met with limited levels of success.[39] In the present meta-analysis, it is found that serum level of α2δ-1 subunit may be a novel diagnostic biomarker which is easy to do and needs further more studies.[40] In another study it is found that α2δ-1 could serve as a new prognostic biomarker.[41]

This meta-analysis shed light on the role of CXCL11, pre-B-cell leukemia transcription factor 3 (PBX3), Islet1 (ISL1), IP3R2-mediated Ca^{2+} oscillation, lysyl oxidase (LOX), Erk, NOTCH3, DNA damage repair proteins, PKM2, and TLR-4 in the context of self-renewal efficiency and tumorigenesis of α2δ-1+ cancer cells.[24,42-50] By meticulously dissecting and elucidating the role of each of these factors, the experiments sought to unravel the complex pathways and molecular interactions that govern the self-renewal process and tumorigenic potential of α2δ-1+ cancer cells. The comprehensive examination of these factors and their impact on the cellular mechanisms underlying cancer development and progression aimed to deepen our understanding of the intricate cellular processes involved in tumor formation and provide valuable insights into potential therapeutic strategies targeting these specific factors. The findings obtained from these experiments are expected to pave the way for future research endeavors, guiding the exploration of novel therapeutic approaches and interventions for the treatment of α2δ-1+ cancer cells.

CXCL11, a chemokine with the ability to bind to two distinct chemokine receptors, namely CXCR3 and CXCR7, has gained significant recognition in current tumor research due to its multifaceted involvement.[51] The wide-ranging functionalities exhibited by CXCL11 encompass the inhibition of angiogenesis, modulation of the proliferation of diverse cell types, participation in the invasion of carcinoma directed by fibroblasts, augmentation of adhesion properties, suppression of M2 macrophage polarization, as well as the facilitation of immune cell migration. The present study mechanistically explores that CXCL11 chemokine plays a crucial role in enhancing the self-renewal capacity and tumorigenic potential of liver TIC that express α2δ-1.[42] This effect is mediated through the activation of the CXCR3 receptor and subsequent activation of the ERK1/2 signaling pathway. This opens a new window in the implementation of targeted CXCL11 therapies.

PBX3, a transcription factor of the pre-B cell leukemia (PBX) family, exhibits the unique ability to establish specific and stable interactions with DNA even in the absence of cofactors. Extensive research has consistently reported PBX3's involvement in the development and maintenance of a malignant phenotype. Moreover, elevated levels of PBX3 tumor expression have been directly associated with decreased overall survival in cancer patients.[52] In light of these findings, this meta-analysis explores the suppressive effects of let-7c, miR-200b, miR-222, and miR-424 on the function of PBX3.[43] Interestingly, these four miRNAs work together to target PBX3, a protein that is both necessary and sufficient for the acquisition and maintenance of TIC properties. Furthermore, PBX3 plays a crucial role in activating a transcriptional program that leads to the expression of genes essential for HCC TIC stemness, including CACNA2D1, EpCAM, SOX2, and NOTCH3. Additionally, the expression levels of CACNA2D1 and PBX3 mRNA can serve as prognostic indicators for poor outcomes in HCC patients. In summary, this study provides a more profound understanding of PBX3's potential as a promising therapeutic target.

Insulin gene enhancer binding protein 1 (ISL1) is a LIM homeodomain transcription factor which has been found to be highly expressed in a variety of malignant tumors.[53] ISL1 gene is known to play a crucial role in the self-renewal and maintenance of TICs. It has been observed, in a related context, that miR-31 possesses the ability to exert a negative regulatory influence on the self-renewal capacity of α2δ1+ liver TICs.[44] This regulatory effect is achieved through the process of silencing the ISL1 gene, which is known to play a crucial role in the self-renewal and maintenance of TICs. The discovery of this regulatory mechanism sheds light on the intricate molecular pathways involved in the regulation of TICs and provides valuable insights into potential targets for therapeutic intervention.

The oscillation of Ca^{2+} is a property that exists at the system level of the cellular machinery responsible for

handling Ca^{2+}, and it encodes various signals that are both physiological and pathological in nature. Sun et al. study aims to test the hypothesis that Ca^{2+} oscillations play a crucial role in maintaining the stemness of CSCs, which are believed to be responsible for the initiation and progression of cancer.[45] Their findings indicate that Ca^{2+} oscillations stimulated by niche factors are a distinctive characteristic of CSC-enriched Hep-12 cells and purified α2δ1+ CSC fractions from HCC cell lines. In Hep-12 cells, the frequency of Ca^{2+} oscillation is positively correlated with the potential for self-renewal. Through the use of a newly developed Ca^{2+} sensor called GCaMP-ER2, which is localized in the endoplasmic reticulum (ER), they have demonstrated that CSCs exhibit distinctive oscillatory release of Ca^{2+} in the ER, which is controlled by the type 2 inositol 1,4,5-trisphosphate receptor (IP3R2). Knockdown of IP3R2 significantly impairs the self-renewal capacity of liver CSCs. Based on these findings, they propose that targeting the IP3R2-mediated Ca^{2+} oscillation in CSCs could provide a novel and physiologically inspired anti-tumor strategy for liver cancer.[45]

Lysyl oxidase (LOX) is an extracellular enzyme that is known to play a critical and essential role in the process of crosslinking collagen molecules.[54] The present meta-analysis demonstrates that α2δ-1^{+} TICs, which are present in HCC, have been observed to exhibit a specific capability of secreting LOX.[46] This particular process, facilitated by the secretion of LOX, leads to the formation of a rigid and inflexible extracellular matrix, which in turn initiates and promotes the development of TICs that display an intrinsic and inherent mechanical stiffness. It is of utmost importance to take note and acknowledge that this particular mechanical characteristic of the TICs is indeed substantial and significant enough to effectively and efficiently facilitate the initiation and subsequent progression of tumor growth within the affected tissues.

The proteins known as extracellular signal-regulated kinase, ERK1 and ERK2, which are members of the mitogen-activated protein kinase (MAPK) family, are integral constituents of a complex phosphorelay pathway.[55] This pathway is responsible for transmitting and transmitting growth and mitogenic signals that are primarily mediated by the small RAS GTPases. The identification of activation and over-expression of Erk in the positive H1048 cell line, specifically in the α2δ1 subpopulation, is successfully accomplished through protein-level analysis.[47] This discovery sheds light on the intricate molecular mechanisms at play within this particular cellular context. Moving forward, it is imperative to delve deeper into understanding the specific role that the MEK/ERK pathway plays in the development of chemoresistance in SCLC. This pathway, known for its involvement in signaling cascades, exhibits a promising potential for therapeutic interventions aimed at overcoming chemotherapy resistance. Consequently, further investigation into the intricacies of this pathway is crucial in order to effectively address the challenges posed by chemoresistance in SCLC.

The receptor known as Notch exhibits transmembrane characteristics and functions as a critical developmental signalling entity that is involved in various fundamental processes such as developmental patterning, determination of cell fate, and the regulation of cell survival and proliferation. One of the four mammalian Notch proteins, Notch3, serves as a signalling receptor that assumes a pivotal role in dictating the fate of cells in numerous developmental scenarios as well as in adult tissue contexts.[56-58] The influence of α2δ1 on the capacities of TICs is subject to the modulation of the Notch signaling pathway. α2δ-1 possesses the capability to regulate the transcriptional activity of NOTCH3, thereby directly impacting the expression of this important signaling molecule.[24] The upregulation of NOTCH3, a crucial component in the Notch pathway, is facilitated through the activation of α2δ-1, which in turn engages the Ca^{2+}-Calcineurin/NFATc2 signaling cascade. This intricate signaling network involving α2δ-1 and Notch signaling pathway components plays a significant role in the regulation of TIC capacities, further highlighting the functional relevance of α2δ-1 in cellular processes.

Cells exhibit a response to genotoxic stress by means of intricate and multifaceted protein pathways referred to as the DNA damage response (DDR). These complex mechanisms are responsible for ensuring the preservation of the cell's genomic integrity, and they are also involved in the activation of processes such as DNA repair, regulation of the cell cycle, and, ultimately, the initiation of programmed cell death.[59] It is important to note that any alterations or modifications within the DDR protein network may give rise to various diseases, with a particular emphasis on the development of cancer. In recent times, researchers and medical professionals have made significant progress in exploring and exploiting the vulnerabilities within the DDR network, effectively utilizing these insights to enhance and optimize cancer treatments through the implementation of DNA damage strategies and the combination of various therapeutic approaches. It has been observed that α2δ-1-high NSCLC cells exhibit resistance to radiation, and it is believed that α2δ-1 contributes to this radioresistance by enhancing the efficiency of DNA damage repair. By specifically targeting α2δ-1-high cells with the monoclonal antibody, the radiosensitivity of these cells can be increased, suggesting that the antibody has the potential to improve treatment outcomes when used in combination with radiation.[48]

Pyruvate kinase M2 (PKM2), an essential enzyme that governs the rate of glycolysis, emerges as a crucial regulator within the intricate realm of tumor metabolism. This particular enzyme, PKM2, has been unequivocally proven to exhibit heightened levels of expression amidst a multitude of cancer types, thus exerting an influential impact on the proliferation and metastasis of malignant

tumor cells.[60] The role of α2δ-1 in promoting the properties of pancreatic TICs involves a series of phosphorylation events on pyruvate kinase M2 (PKM2), which are mediated by calcium/calmodulin-dependent protein kinase II delta (CaMKIIδ). The phosphorylation of PKM2 is a crucial step in the activation and regulation of its function within TICs. Specifically, α2δ-1 acts as a facilitator in this process, promoting the sequential phosphorylation of PKM2 by CaMKIIδ. Through this mechanism, α2δ-1 plays a key role in enhancing the properties of TICs in PC. Consequently, targeting α2δ-1 emerges as a promising therapeutic strategy to counteract the tumorigenic effects of TICs in pancreatic cancer. By disrupting the α2δ1-mediated phosphorylation of PKM2, this therapeutic approach aims to impair the functional capabilities of TICs and hinder their ability to drive tumor progression and metastasis. Overall, the identification of α2δ-1 as a critical regulator of TIC properties and the development of a targeted therapeutic strategy against α2δ-1 provide valuable insights and potential interventions in the context of PC.[49]

TLR-4, a member of the toll-like receptor family, is known for its crucial role in the innate immune response.[61] The activation of TLR-4 plays a pivotal role in the regulation of α2δ-1 expression, a protein involved in various physiological processes.[50] This regulation occurs through the intricate NF-kB/Sp1 signaling pathway, a complex network of molecular interactions and signal transduction mechanisms. Upon activation, TLR-4 triggers a cascade of events that ultimately lead to the modulation of gene expression, including the upregulation of α2δ-1. The NF-kB/Sp1 signaling pathway, consisting of the nuclear factor-kappa B (NF-kB) and specificity protein 1 (Sp1), is responsible for transducing the signals initiated by TLR-4 activation to the nucleus. NF-kB, a transcription factor, is activated upon TLR-4 stimulation and translocates to the nucleus, where it binds to the promoter region of the α2δ-1 gene. This binding event leads to the recruitment of various coactivators and transcriptional machinery, resulting in the enhanced expression of α2δ-1. Additionally, Sp1, another transcription factor, interacts with NF-kB to further regulate the transcriptional activity of the α2δ-1 gene. The intricate interplay between NF-kB and Sp1 in the context of TLR-4 activation ensures precise control over α2δ-1 expression, facilitating its involvement in diverse cellular processes.[50] Overall, the regulation of α2δ-1 expression through the NF-kB/Sp1 signaling pathway provides valuable insights into the intricate mechanisms underlying immune responses and cellular physiology.

Thus, the present study unveils and brings to light the important finding that the cancer cells, which possess and express the specific characteristic of α2δ-1, possess and exhibit the inherent potential and capability to function and operate as noteworthy, significant, and valuable cell surface markers for the tumour-initiating cells, also commonly referred to as cancer-initiating cells, as well as the cancer stem cells, which are acknowledged and recognized as the driving force behind the initiation, progression, and development of cancer, thereby emphasizing and underscoring their pivotal and critical role in the intricate and complex tumorigenic process.

This particular study, much like numerous other studies conducted in the field, does not come without its fair share of limitations. As a study that conducts a meta-analysis on the specific topic of the involvement of α2δ-1 in the process of oncogenesis, the authors find themselves restricted by the limited number of participants included in the study. Furthermore, the search approach employed in this study is purposely confined to the utilization of the PubMed database exclusively, thereby limiting the scope of available data. In addition to this, a relatively narrow range of methodological designs were employed in this study. Consequently, it is reasonable to suppose that a certain portion of the existing literature on this subject matter may not have been considered or incorporated into our meta-analysis.

CONCLUSION

In the span of twenty-four years, a mere seventeen experiments have been conducted that specifically investigate the involvement and significance of α2δ-1 in the complex process of oncogenesis. The scarcity of experiments dedicated to studying the multifaceted role of α2δ-1 in oncogenesis highlights the current gaps in our understanding and motivates the need for more comprehensive and in-depth investigations. It is crucial to prioritize and invest in further research endeavors that address the role of α2δ-1 in oncogenesis, in order to broaden our understanding of this intricate molecular pathway and harness its potential for the development of innovative cancer treatments.

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