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Review Article

Navigating Gastrointestinal Cancer: The Role of DCLK1, the Tumor Microenvironment, and Advanced Immunotherapies

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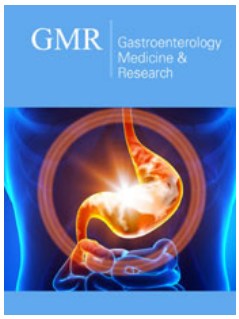

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Abstract

Doublecortin-Like Kinase 1 (DCLK1) is an important cancer stem cell marker associated with tumor progression and metastasis in gastrointestinal, pancreatic, breast, lung, and ovarian cancers. DCLK1 is an important key molecule for the initiation, promotion, metastasis, and development of therapy resistance in cancer. Various signalling molecules, epigenetic changes, hypoxia, and Epithelial-To-Mesenchymal Transition (EMT) can promote DCLK1 expression in the tumor microenvironment. Accumulating reports suggest that DCLK1-targeted immunotherapies play an important role in controlling the proliferation of Cancer Stem Cells (CSCs) and inhibiting tumor proliferation in cancer. DCLK1-targeted immunotherapies, such as monoclonal antibodies, small molecule inhibitors, and engineered immune cells, may play a role in tumor regression. This review elucidates DCLK1-targeted immunotherapies play an important role in controlling the proliferation of CSCs and cancer treatment.

Keywords: DCLK1; Cancer stem cells; Immunotherapy; Cancer; CAR-T cells; Monoclonal antibodies

Introduction

Gastric Cancer (GC) is the second most commonly diagnosed cancer and the fourth leading cause of death due to progression [1]. More than 1 million people are newly being diagnosed each year [2]. The risk factors may depend on physical and chemical characteristics of the human body, such as age, gender, and ethnicity or origin, from which the person prevails [2,3]. Other factors include dietary habits, smoking, and genetic susceptibility. Men are more likely to be affected than women [2]. Most cases occur in Asia and South American countries [3]. The highest mortality rate is recorded in East Asia [2]. According to the WHO classification, gastric cancer consists of four subtypes: papillary, tubular, signet ring, and mucinous [3]. Doublecortin-Like Kinase 1 (DCLK1) is a microtubule-associated, bifunctional protein [4,5]. It is a member of the Mitogen Associated Protein Kinase (MAPK) [4,5]. It is mainly found in the tuft cells of the gastrointestinal tract [4]. It is also expressed in the heart, lung, spleen, thymus, prostate, and intestine [6]. It is important for the survival rate of patients suffering from GC. The protein structure consists of a C-terminal serine/threonine kinase domain and an N-terminal region consisting of DCX1 and DCX2 motifs [4,7]. DCX1 and DCX2 bind mainly to microtubules and their dimers [6]. The N-terminal part regulates microtubules polymorphism, while the C-terminal part facilitates interactions between several proteins [4]. This prognostic cancer cell marker is present in malignant Gastrointestinal (GI) tumors and cancer-initiating cells [4]. When the expression of DCLK1 is upregulated in GI cancers, increased mortality and recurrence are observed [7]. This downregulates microRNA and upregulates transcription factors associated with Epithelial-to-Mesenchymal Transition (EMT), which promote angiogenesis and metastasis [4]. The expression of DCLK1 is mainly regulated by alternative promoter usage, gene splicing or post-transcriptional cleavage [5]. The expression of DCLK1 correlates with the expression of immune cell markers, such as

monocytes and macrophages that downregulate $CD8^+$_ T cells, which weakens immune cell function [7].

GC is an example of a complex and dynamic tumor that is influenced by the Tumor Microenvironment (TME) [8]. It consists of a variety of cellular components (cancer cells, immune cells, fibroblasts, and endothelial cells) as well as the Extracellular Matrix (ECM) [9]. Therefore, the elucidation of the TME is necessary for the individualization of therapeutic regimens and the prediction of prognosis in gastric cancer [9]. The immune cell infiltrate is one of the most important attributes of the TME, which features Tumor-Infiltrating Lymphocytes (TILs), Tumor-Associated Macrophages (TAMs), and Cancer-Associated Fibroblasts (CAFs) [8]. In gastric cancer, TILs play a dual role by exerting both antitumor and immunosuppressive functions [9]. The TME is indeed not simply an inert backdrop, but actively contributes to cancer progression via various mechanisms [8]. One important feature is immune evasion, i.e., the TME creates an immunosuppressive environment in which cancer cells can avoid immune recognition [8]. TME has been shown to promote metastasis by creating a favorable environment for cancer cells to invade surrounding tissues and spread to distant sites [8]. Cancer cells in the exudate induce a TME characterized by hypoxia and inflammation. For example, malignant ascites from advanced gastric cancer supports peritoneal metastasis [9]. The TME not only confers resistance to standard treatments, but also protects the tumor and accelerates resistance to immunotherapies [9]. The exact composition of the presence of TME immune cells presence and the correctness of a variety of immune cell types in the TME could be biomarkers for factors that influence treatment efficacy, so that personalized intervention strategies are increasingly needed [9].

Several studies conducted in recent years have shown that the characteristics of TME can be utilized as predictive markers in GC [8]. Thus, a higher TME score, indicative of immune activation, is associated with better survival, while a low TME score, associated with T-cell suppression, is associated with poor prognosis [8]. Understanding these dynamics may help in the selection of patients who may benefit from immunotherapy and other targeted treatments [10]. By molecularly analyzing the communication of cancer cells with their environment, more targets can be identified for a therapeutic approach and better prediction of the disease to enhance the treatment of GC [11].

The treatment method, in which genetically modified immune cells reshape the immune response to selectively target malignant cells, is revolutionary [12]. CAR-T cells are engineered to express chimeric antigen receptors that can specifically recognize different antigens that are only expressed on tumor cells [12]. These cells have achieved considerable success in hematologic malignancies, but their performance in more solid tumors has been much lower [13]. The major challenge in solid tumors is the immunosuppressive TME along with physical barriers that prevent CAR-T cells from entering and killing target cancer cells [14,15]. Therefore, there are currently a number of approaches, such as CAR-T with higher survival rates compared to TME or in combination with checkpoint inhibitors and also in combination with chemotherapy and immunotherapy [16,17]. Alternatively, TCR T cells could target antigens presented as neoantigens- new proteins unique to cancer cells- by a much larger number of tumor cells [18]. This therapy could work particularly well in solid tumors, where diversity of antigens is the biggest problem [18]. However, it carries risks in the form of TCR mismatch and cytokine storms, which should be treated with caution [18]. Another promising CAR-T targeted therapy is CAR-T targeting DCLK1, but this is likely to prove particularly effective in colorectal cancer as this compound is reported to be highly overexpressed in CSCs [19]. CSCs are responsible for cancer progression and metastasis. Therefore, they represent important targets for therapies aimed at eliminating CSCs and thus reducing the likelihood of recurrence and resistance [19,20].

One of the most important classes of immunomodulators, such as PD-1/PD-L1 inhibitors, acts by blocking negative signals that limit T-cell assault on cancer [21]. DCLK1 plays a role in upregulating the surface expression of PD-L1 on tumor cells and may be associated with various tumor immune defense strategies [21]. The combination of CAR-T or TCR-T therapies with immune checkpoint inhibitors appears to increase the therapeutic help in solid tumors [14,22]. LRRK2-IN-1, a small molecule inhibitor, acts specifically on the protein leucine-rich repeat kinase 2 [5]. It is significantly involved in the regulation of immune cells and autophagy. Its main effect was first described in neurodegenerative diseases, but its importance for cancer immunotherapy is increasing rapidly. LRRK2-IN-1, via LRRK2, generally modulates immune responses by decreasing immunosuppression within the TME to enhance anti-tumor immunity. Second, LRRK2-IN-1 may also block autophagy, which is utilized by cancer cells for survival [5,23]. Since the suppression of LRRK2 is specific, LRRK2-IN-1 can potentially be used in combination with cancer [23]. The future of engineered immune cell therapy will depend on solving these challenges increasing antigen specificity, T-cell longevity, and optimizing combination strategies to enable more effective treatments for both hematologic and solid malignancies.

Tumor microenvironment

The tumor cell environment is referred to as the TME, which includes both cellular and acellular components [24]. The TME includes the Extracellular Matrix (ECM), blood and lymphatic vessels, stromal cells, immune cells, and other cell types [25]. Immune cells in the TME, such as Tumor-Associated Macrophages (TAMs), play a number of roles in cancer biology, including promoting tumor cells and facilitating the support for angiogenesis [25]. TAMs constitute the majority of cellular components in the TME and are polarized to promote or suppress tumorigenesis [25]. Tumor-Associated Neutrophils (TANs) enhance tumor metastasis and aggressiveness by promoting the EMT process [25]. The chemokine CXCL5 secreted by TANs is a marker of tumor progression and can potentially be considered a critical target for inhibiting metastasis [26]. CXCL5 can activate neutrophils and GC [25]. Remodelling of the ECM by Cancer-Associated Fibroblasts (CAFs) facilitates the invasion potential of cancer cells in the stroma

for the temporal dynamics of tumor malignancy [27]. Control of immune checkpoints, particularly PD-1 and PD-L1, has revealed significant opportunities for augmenting the immune responses against gastric cancer [24]. Although Immune Checkpoint Blockade (ICB) therapies, such as anti-PD-1 and anti-PD-L1 antibodies, have led to remarkable results in some cancers, objective clinical response rates of ICB in advanced GCs are unreliable [28]. More conventional biomarkers, such as PD-L1 CPS and MSI-Hand TMB, have significant limitations as they primarily reflect tumor cell characteristics and do not take into account the crucial influence of the TME [28]. In addition, several other markers (e.g., CD10, FAP, and GPR77) are associated with aggressive form factors and adverse prognosis-related outcomes in GC treatment [26]. The success of immunotherapy, especially in $CD8^+$ T-cell function, depends on a thorough understanding of the TME and the dangers immune cells face when they come into contact with macrophages recruited to TAMs [27].

DCLK1 is overexpressed in GC, leading to poor prognostic outcome and chemoresistance [26]. DCLK1 maintains the viability of CSCs, which proliferate and promote tumorigenesis [25]. DCLK1 promotes the survival and proliferation of these cells [27]. In addition, to targeting DCLK1 as described above, we also reasoned that this gene may be responsible for the stem cell-like properties of the cells, making them more susceptible to treatment [26]. DCLK1 is of great research interest area due to its ability to maintain stem-like properties and resistance [26]. One of the possibilities is to produce a global therapeutic approach for the treatment of GC, which will lead to more studies based on combined therapies [27]. Therefore, further research is needed to devise appropriate combination treatment regimens that can help patients [26]. The association between the expression of DCLK1 and the components of TME remains consistent across different cancer stages, indicating its significant role in tumor progression [27]. Suppression of DCLK1 may act synergistically with conventional therapies and allow overcoming drug-resistant phenotypes [27]. One area of high research interest that has been targeted is DCLK1 due to its ability to maintain stem-like properties and resistance [26]. The TME influences CSC behavior by secreting cytokine and activating signalling pathway that promote CSC invasion, metastasis, and resistance to therapy [24]. Most cancer immunotherapy strategies aim to overcome the obstacles of the TME to achieve therapeutic effects [29]. CSCs can remodel their microenvironment to create a niche favors their survival and stem cell formation [24]. One of the major obstacles is the TME, which greatly hinders immunotherapies and therefore necessitates the development of new therapeutic strategies [29]. Targeting specific TME components, such as CAFs, TAM, and m6A regulatory protein, could be a niche to improve GC treatment [25]. Enzymes and signalling pathways involved in ECM modification could be considered novel therapeutic interventions for GC. This approach targets the structural and functional changes in the ECM that promote tumor growth and resistance [26]. Different mechanisms of cell death in combination with traditional therapies and natural products open new avenues to achieve synergistic effects [27]. The development of tactics that emphasize the unique properties of the gastric TME, particularly by targeting specific signalling pathways, is recognized as a viable avenue for therapeutic solutions [24].

Engineered immune cells for cancer therapy

Artificially engineered immune cells are the new and emerging technology in the world of cancer treatment. One of the best examples is the chimeric antigen receptor [12]. These CAR-T cells are collected from each individual and remodeled according to the binding sites of the specific proteins on the surface of the cancer cells. Owing to this property, CAR-T cells are often referred to as an animated panacea [12]. In the past, this personalized CAR-T cell therapy was not efficient because it only targeted liquid tumors. The solid tumors have a TME that is excluded from the immune system. This suppresses the immune response of T cells. However, due to recent discoveries, antigen-presenting cells such as dendritic cells and macrophages are used as a pro-inflammatory immune response to remodel them from immune-excluded to immune-included [14]. To achieve high efficiency of CAR-T cells, we need a larger number of them. This can be achieved by culturing the cells and modifying them to express the CAR-T. This process can take up to 5 weeks [12]. Therefore, patients suffering from aggressive cancers may not opt for this intravenous treatment and the cancer metastasizes quickly [12]. Despite the revolutionary effect of CAR-T cell therapy in blood cancer, its application in solid tumors faces enormous challenges [13]. The immunosuppressive TME, which is characterized by regulatory T cells, immune checkpoint molecules, and inhibitory cytokines, dampens the activity of CAR-T cells [13]. In addition, the dense ECM and abnormal vasculature of solid tumors act as physical barriers that impede T cell infiltration and access to tumor cells [22]. In addition, the lack of truly tumor-specific antigens and the great heterogeneity of solid tumors, in which antigen expression varies from cancer cell to cancer cell and may even disappear during progression, pose significant hurdles to targeted therapy [30]. However, there are promising strategies to overcome these challenges [17]. Manipulation of the TME involves the use of monoclonal antibodies to eliminate T-regs, blocking immune checkpoints, and engineering CAR-T cells to express molecules that attract the immune system [13,17]. Engineering the cells using genome editing tools can enhance their resistance to the immunosuppressive environment and exhaustion [13,17]. Combination therapies with chemotherapy, anti-angiogenic agents or immunomodulatory approaches such as cancer vaccines or photothermal therapy can improve the migration, proliferation, and anti-tumor activity of CAR-T cells [13,16]. Finally, the search for novel tumor-specific antigens, such as glycosylated antigens or tumor-specific mutations, offers the hope of overcoming the limitations of existing targets [4]. Although challenges remain, these diverse strategies hold promise for unlocking the full potential of CAR-T cell therapy in the fight against solid tumors [13].

The other new immunotherapy is T-Cell Receptor (TCR) targeting. This involves equipping T cells with newly created TCRs that are specifically designed to recognize certain tumor antigens. This enables the T cells to find and destroy cells with

high specificity [31]. Although TCR T-cell therapy is a promising approach to support the immune system in the fight against cancer, there are still some difficult hurdles to overcome in translating it into a clinical therapy [18]. A critical challenge lies in a potential TCR mismatch, where the introduced receptors could attack healthy tissue and mimick the life-threatening graft-versus-host disease [18]. This can be mitigated by silencing endogenous TCRs using genetic tools or by using alternative cell sources such as γδ+ T cells. Another hurdle is non-specific cytotoxicity, where apparently specific TCR T cells can attack healthy tissue expressing similar antigens [18]. This requires meticulous selection of tumor-specific antigens with minimal expression in healthy organs or the exploitation of tumor-specific mutations (neoantigens) for TCR design. In addition, TCR-T therapy can trigger a potentially lethal cytokine storm [18]. Preemptive reduction of tumor burden and development of strategies to control cytokine release are critical to address this concern. Finally, hostile TME, characterized by limited access to T cells, immune exhaustion, and immunosuppressive cells, is a significant barrier [18]. Combination therapies with agents such as checkpoint inhibitors or strategies to modulate the TME offer promising solutions to overcome this hurdle. Several challenges remain, but persistent efforts to find innovative solutions involving precise target selection, advanced genetic engineering, TME modulation, and cytokine control are paving the way to unlock the full potential of TCR T cell therapy as a transformative weapon in the fight against cancer.

TCR T cell therapy has ignited hopes for the treatment of solid tumors and has proven its potential in preclinical and clinical studies. Unlike CAR-T cells, which target surface antigens, TCR-T cells can recognize and eliminate cancer cells based on unique "neoantigens" found within them, providing a broader repertoire of targets and potentially reducing off-target effects [18]. Advances in tumor immunology and technologies such as immunome library sequencing enable the identification of these patient-specific neoantigens and pave the way for the development of personalized TCR-T cells [18]. Although challenges remain in manufacturing, managing side effects, and overcoming immunosuppressive TMEs, ongoing research promises that this approach will revolutionize immunotherapy for solid tumors [18]. By leveraging personalized medicine and cutting-edge technologies, researchers are paving the way to a future in which TCR-T cell therapy enables the immune system to target solid tumors with unprecedented precision and efficacy [18]. Programmed cell death ligand-1 is a protein that keeps the body's immune response under control. It is found in some normal cells and also in cancer cells. These proteins help to repair the DNA in tumor cells, which leads to an impairment of the effectiveness of tumor therapy [32]. Therefore, they are the target of immune checkpoint blockade, which represents a major revolution in cancer treatment [32].

CAR-T cell therapy

Revolutionizing the treatment of blood cancers, CAR-T cell therapy harnesses the power of your immune system to target and eliminate cancer cells similar to a precision medicine [19]. Hematologic malignancies are treated with immunotherapy using CAR-T, but treatment of solid tumors has been shown to be less effective [19]. T cells have been modified to activate, proliferate, and eliminate cells [19]. A hypoxia TME lowers the viability of $CD8^+$ cytotoxic T cells [20]. This condition enables the growth of solid tumors and thus increases resistance to therapy [20]. Systemic CAR-T therapy bypasses the immunosuppressive and physical barriers present in solid tumors, contributing to its success in hematologic malignancies [19]. A patient's $CD8^+$ T cell population is harvested and experimented with to produce new chimeric T cells as part of a new treatment, known as CAR-T therapy [19]. The efficacy of second-generation CAR-T cells is being tested in solid tumors, such as advanced sarcoma, liver metastases, mesothelioma, ovarian cancer, and pancreatic cancer [15].

CAR-T cells against gastrointestinal tumors

Clauding 18.2-specific targeted CAR-T cell therapies are promising and have been shown to be effective in phase I clinical trials in GC patients [33,34]. In the treatment of Colorectal Cancer (CRC), therapies today focus on DCLK1 isoforms 2 and 4, which show increased expression in cancer cells [19]. The monoclonal antibody CBT-15 and its humanized derivative, hCBT-15, specifically target these isoforms. In particular, HT29 CRC cells cultured in 3D matrices show a marked upregulation of DCLK1 compared to conventional 2D cultures [19]. This finding suggests that DCLK1 is a potential marker for CSCs due to the increased clonogenic capacity of HT29 cells. Conversely, the HCT-116 and LoVo cell lines have similar DCLK1 levels in both 2D and 3D cultures, indicating possible heterogeneity of DCLK1 expression in different CRC subtypes. Given the significant upregulation of DCLK1 in HT29 cells grown in 3D, which is more consistent with a TME, therapeutic strategies targeting DCLK1 with hCBT-15 or DCLK1-based CAR-T cells appear to be particularly promising for CRC, especially in situations where CSC-like populations predominate [19]. In studies evaluating the efficacy of DCLK1 CAR-T cells against CRC cells, the cells efficiently killed CRC cells in 2D cultures, especially at a higher attacker-to-target ratio (20:1 compared to 10:1) [19]. At this ratio, they destroyed almost all HT29 cells, 60% of HCT116 cells, and 78% of LoVo cells. When exposed to CRC cells, DCLK1 CAR-T cells released significantly more interferon-gamma (IFN-γ) than control cells, confirming their binding to the target cancer cells and their cytotoxic activity. This was observed in both 2D and 3D cultures for HT29 and HCT116 cells. Interestingly, LoVo cells showed a less specific IFN-γ response, with some release even in control cells. This suggests possible factors unique to LoVo cells that influence the results [19].

CAR structure

CARs are known as synthetic receptors. They have an extracellular target antigen-binding domain, a hinge transmembrane domain, and one or more intracellular signalling domains [35].

The antigen-binding domain: It acts like a lock and key, recognizing and binding to a unique marker on the cancer cell. The antigen-binding domains are derived from the chains of monoclonal

antibodies connected by a linker to scFv [36]. To increase the affinity of CARs to their target antigen, factors such as the location of the epitope, the avoidance of scFvs with ligand-independent tonic signalling, and the density of the target antigen can be altered [35].

The hinge region: It is a flexible spacer which ensures that the binding domain reaches its target and overcomes barriers. It allows the antigen-binding domain to access the target epitope [35]. The different length and composition of the hinge region affects the functions of the CARs. The hinge regions are usually derived from amino acid sequences from CD8, CD28, IgG1, or IgG4. The IgG-derived hinge regions can deplete the CAR-T cell, reducing persistence *in vivo*, as they interact with Fcγ receptors [35].

Transmembrane domain: This region helps anchor the CAR to the T-cell membrane and is important for CAR-T cell functions [37]. The CAR transmembrane domains are responsible for this and influence CAR expression levels, stability, and synapse formation [35]. They are derived from proteins such as CD3ζ, CD4, CD8α, and CD28. Overall, the transmembrane domain and hinge region have effects on the cytokine production and Activation-Induced Cell Death (AICD) [35]. The CAR-T cells with CD8α transmembrane and hinge domains release low levels of TNF and IFNγ and are insensitive to AICD compared to CARs with CD28 domains [38]. It has been investigated that CAR expression and stability can be increased by using the CD8α or CD28 transmembrane domains [35].

Intracellular signalling domain: This region plays a pivotal role in controlling the T-cell response to cancerous targets. Therefore, optimization of this domain is the goal of CAR engineering by refining the design and achieving better clinical results with each generation [35]. The first-generation CAR, developed in the late 1990s, had a CD3ζ or FcRγ signaling domain [39]. This resulted in limited efficacy and persistence due to insufficient T-cell activation [40]. In the second generation, CD28 or 4-1BB co-stimulatory domains were introduced in addition to CD3ζ and the importance of co-stimulation in enhancing T cell responses was known [41,42]. In trials, second-generation CAR-T cells have shown strong therapeutic responses in hematologic malignancies [27]. To achieve better results, third-generation CARs incorporated two co-stimulatory domains with CD3ζ and yielded mixed results [39]. The CARs containing CD28 and 4-1BB produced cytokines at good levels in lymphoma and lung metastases and showed improvement in antitumor response [38]. In pancreatic cancer models, third-generation CARs showed no benefits in *in vivo* treatment [43].

CAR-T therapy targeting DCLK1 offers a different approach to cancer treatment by directly targeting CSCs. In contrast to conventional therapies that primarily target bulk tumor cells, DCLK1-CAR-Ts exploit the highly specific expression of DCLK1 on CSCs, minimizing unwanted off-target effects on healthy stem cells. This targeted elimination of CSCs has the potential to disrupt EMT, a process that promotes tumor metastasis and drug resistance. Consequently, DCLK1-CAR-T therapy could not only cause tumor shrinkage but also significantly improve overall survival by preventing disease recurrence and metastasis. This novel strategy represents a significant step forward in addressing the root cause of cancer progression and holds promise for improving patient outcomes [19].

Monoclonal antibody therapy

DCLK1 has been associated with vital functions in cancer, immunology, and even surprising connections to cardiovascular health [7,44,21]. The expression of DCLK1 is evident in a wide variety of cancer landscapes, ranging from the complicated canvases of colon, pancreatic, and esophageal cancers [7]. The unique feature of DCLK1 lies in its association with DCLK1+ cells, robust entities that are resistant to chemotherapy and exhibit an amazing capacity for self-renewal [7,44,45]. The genomic tapestry of DCLK sequences unfolds many variants that represent long or short isoforms in human neurons [19,44,45]. DCLK-1, which is considered as a distinctive marker for certain cancer cells, plays a pivotal role in the progression of various cancers [7,44]. However, methods to target DCLK1 face hurdles such as non-specific binding and instability, which led to the development of monoclonal antibodies (mABs), such as DCLK1-87 mAb and DCLK1-44 mAb [44]. These mAbs, which are characterized by their specificity in binding to DCLK1 in CRC cells, offer potential treatment options [7].

The impact of DCLK1 extends beyond cancer initiation and progression to include epigenetic changes in the promoter region that profoundly affect kidney and breast cancers [44]. Furthermore, it plays an important role in tissue repair and tight junction the maintenance, contributing significantly to decelerating tumor progression [44]. The presence of four isoforms, with isoforms 2 and 4 playing an important role in cancer studies, makes the structure of DCLK1 even more complex [19]. Monoclonal antibodies such as CBT-15 have been developed to recognize DCLK1 sequences and have shown efficacy in arresting xenografts and inhibiting EMT in renal cancer cells [19]. The humanized version of CBT-15, hCBT-15, extends its reach by recognizing specific domains of DCLK1, demonstrating its potential in cancer treatment [19]. Elevated DCLK1 levels correspond with worsening of the condition, affect EMT-related transcription factors, and influence the response to immune checkpoint inhibitors [19]. This increases EMT-related transcription factors, such as SNA1, ZEB1, and ZEB2, leading to the expression of cell death ligands, such as PD-L1 and PD-L2 mRNAs [19]. The response to immune cell inhibitors increases. T cells are induced to recognize target sites by the expression of tumor-specific antigens [19]. Adoptive T cell-based immunotherapy, a paradigmatic approach, shows promise in targeting tumors and their microenvironment, particularly in pancreatic cancer xenograft models [19]. The intricate involvement of DCLK1 in KRAS activation underscores its importance in cancer development [46]. Expression of DCLK1 activates KRAS, an oncogene that can become a cancer cell when mutated. Its regulatory role in signalling pathways associated with cancer growth and stem cell formation, as well as its involvement in the TME, make DCLK1 an indispensable marker in GC [7,19,46].

Beyond its prominent role in cancer biology, DCLK1 is closely intertwined with the immune response [44]. Its expression serves

as an independent predictor of poor disease-specific survival, with low expression correlating with shorter survival in colon adenocarcinoma [25]. The association of DCLK1 with various immune cells, including T cells, B cells, monocytes, Tfh, Treg, and M2, highlights its complex involvement in modulating the immune response to cancer [25]. In GI cancers, DCLK1 expression is closely associated with inflammation, tumor development, and EMT [25,19]. The balance between pro-inflammatory M1 and anti-inflammatory M2 macrophages, in conjunction with $CD8^+$ T cells, plays a critical role in regulating the immune response in the GI tract [31]. Non-coding RNAs, especially miRNAs, contribute significantly to cancer pathways, highlighting the interplay between DCLK1 and the immune system [19]. M2-like TAMs (tumor-associated macrophages) induce factors that inhibit the infiltration of $CD8^+$ T cells, contributing to the TAM development [25]. Although there is a weak correlation between DCLK1 expression and infiltration of $CD8^+$ T cells, infiltration of M2 macrophages is high. M2 macrophage markers, such as VSIG4, CD163, and MS4A4A, show a strong association with DCLK1 [25]. Expression of DCLK1 activates increased production of Treg and depletion of T cells, demonstrating its profound impact on the immune landscape in cancer [25].

Surprisingly, the influence of DCLK1 extends beyond cancer and immunology, as recent research reveals its intriguing links to obesity-related cardiovascular problems [45]. Obesity, which is characterized by excessive or disproportionate accumulation of fat, poses a serious threat to cardiovascular health [45]. Overexpression of DCLK1 has been found in cardiac tissue of obese mice, suggesting a possible involvement in cardiomyopathy [45]. Studies in which DCLK1 was knocked down in mice, particularly in macrophages, have shown that heart problems triggered by a high-fat diet can be prevented [45]. This prevention includes dysfunction, cardiac hypertrophy, and the excessive formation of fibrosis [45]. These findings emphasize the role of DCLK1 in activating RIP2/TAK1 and triggering inflammatory responses in macrophages when exposed to a high-fat diet or fatty acid [45]. The use of a pharmacologic inhibitor of DCLK1 has shown promise in protecting the hearts of mice exposed to a high-fat diet, suggesting that DCLK1 is a potential target for therapeutic intervention in obesity-related cardiovascular problems [45]. In this context, the pro-inflammatory mechanism of DCLK1 opens new avenues for research and potential treatments, bridging the gap between cancer biology and cardiovascular health [45].

Small molecule inhibitors and immune modulators for cancer therapy

PD-1 or programmed death-1 immunotherapy is a cancer therapy with promising clinical success [21]. It is an inhibitory receptor on the surface of T cells whose main function is to downregulate T cells by activating the TCR signalling cascade [21]. PD-1 is a transmembrane protein that belongs to the CD28 superfamily [21]. Expression of PD-1 is often observed in various types of cancers, such as GC, melanoma, and hepatoma [21]. DCLK1 contributes to the upregulation of PD-L1 expression in cancer cells by regulating Yes-Associated Protein (YAP) in the Hippo signalling pathway [22]. The Hippo signalling pathway is critical for the regulation of cell proliferation and is associated with the development and progression of various diseases, including cancer [47]. This signalling pathway consists of several key components, including mammalian Ste20-like kinases 1/2 (MST1/2), large tumor suppressor 1/2 (LATS1/2), YAP transcriptional coactivator with PDZ-binding motif (TAZ), also known as WW domain-containing Transcriptional Regulator 1 (WWTR1) [47]. These components modulate cell growth and tissue homeostasis [47]. When the Hippo signalling pathway is dysregulated, this can lead to abnormal cell proliferation and contribute to the development of diseases such as cancer [47]. Blocking the interaction of PD-1 interaction with PD-L1 (Programmed death ligand-1) can terminate the PD-1-mediated signalling pathway, activate anti-tumor responses, and improve a patient's survival rate [21]. Since the use of mAb in PD-1 is limited due to immune side effects, small inhibitor molecules are used [21]. Small inhibitor molecules block PD-1/PD-L1 interactions when they interact with immune checkpoints [21]. Small inhibitor molecules are effective in tumor penetration and oral bioavailability [21]. Synthetic small molecules such as Bristol Myers Squibb and Curis Inc can suppress tumor growth and progression [21].

LRRK2-IN-1 is a small molecule kinase inhibitor that improves the condition of cancer patients [5]. It showed selective affinity for DCLK1 and inhibits the function of DCLK1 [5]. This suggests that DCLK1 kinase is a promising anticancer target [5]. To test this, kinome profiling was performed to verify the inhibition of DCK1 kinase by LRRK2-IN-1. Since DCLK1 is a member of the calmodulin-dependent protein kinase, many structures are solved [23]. The docking site with the highest affinity for LRRK2-IN-1 is located at the ATP-binding docking site of the DCLK1 structure, which is very close to the hinge of the kinase and interacting residues [23]. This inhibits DCLK1 kinase activity by not allowing ATP to bind to the binding pocket of DCLK1. LRRK2-IN-1 has a cytotoxic effect on pancreatic cancer cell lines, such as AsPC-1 [23]. LRRK2-IN-1 induces G1 and G2 cell cycle arrest, thereby reducing cancer cell proliferation and viability [23]. The concentration of LRRK2-IN-1 determines the cell cycle inhibitory factor, i.e., lower concentrations induce the G1 cell cycle and higher doses induce the G2/M cell cycle [23]. It is also observed that LRRK2-IN-1 treatment activity inhibits DCLK1 mRNA function and protein expression [5]. Stem cell markers, such as LGR5 and BMI1, which are expressed in DCLK1 in intestinal cells, lead to adenomas and adenocarcinomas of the pancreas after radiation damage [23]. A downregulation of LGR5 and BMI1 mRNA expression was observed after LRRK2-IN-1 treatment [23]. LRRK2-IN-1 was found to have anti-stem cell properties [5]. Stem cell-associated gene expression and pluripotency were reduced upon this treatment by downregulation of LGR5 and BMI1 mRNA [23]. The primary trigger of PDAC, the KRAS oncogene, is also thought to help the disease by downregulating the oncogene in AsPC-1 cells [23]. LRRK2-IN-1 upregulates E-cadherin, an epithelial marker, and downregulates EMT factors. YAP 1 controls KRAS-independent tumor maintenance [23]. The Yap1/Tead2 complex collaborates with the transcription factor E2F, which is involved in the cell cycle and DNA replication program [23].

Conclusion

This review highlights the multifaceted role of DCLK1 in cancer biology, with a particular focus on its importance in gastric cancer progression, metastasis, and resistance to therapy. As a CSC marker, DCLK1 orchestrates key processes such as EMT, immune evasion, and TME modulation, making it an important target for innovative cancer treatments. Overexpression of DCLK1 in CSCs not only drives tumor growth and metastasis, but also contributes to therapy resistance, underscoring its role as a critical determinant of poor prognosis. The TME, characterized by its immunosuppressive milieu and physical barriers, represents a significant obstacle to effective therapy, especially in solid tumors. This review highlights the interplay between DCLK1 and various TME components, including Tumor-Associated Macrophages (TAMs), Cancer-Associated Fibroblasts (CAFs), and immune checkpoints such as PD-1/PD-L1, which together enhance immune evasion and resistance to therapy. However, advances in immunotherapy, particularly the development of immune cell therapies such as CAR-T and TCR-T cells, are changing the landscape of cancer treatment. CAR-T cells targeting DCLK1 have shown remarkable potential in selectively eradicating CSCs, disrupting EMT, and preventing tumor recurrence while minimizing off-target effects. These approaches represent a paradigm shift by addressing the root causes of cancer progression rather than just the tumour bulk. Complementary strategies such as monoclonal antibodies (e.g., DCLK1-specific mAbs) and small molecule inhibitors (e.g., LRRK2-IN-1) offer additional therapeutic options. These modalities have shown promise in inhibiting EMT-related pathways, modulating the TME, and sensitizing tumors to conventional therapies. The integration of these targeted therapies with immune checkpoint inhibitors and other conventional treatments holds the potential to overcome the challenges posed by tumor heterogeneity and resistance mechanisms (Figure 1 & 2).

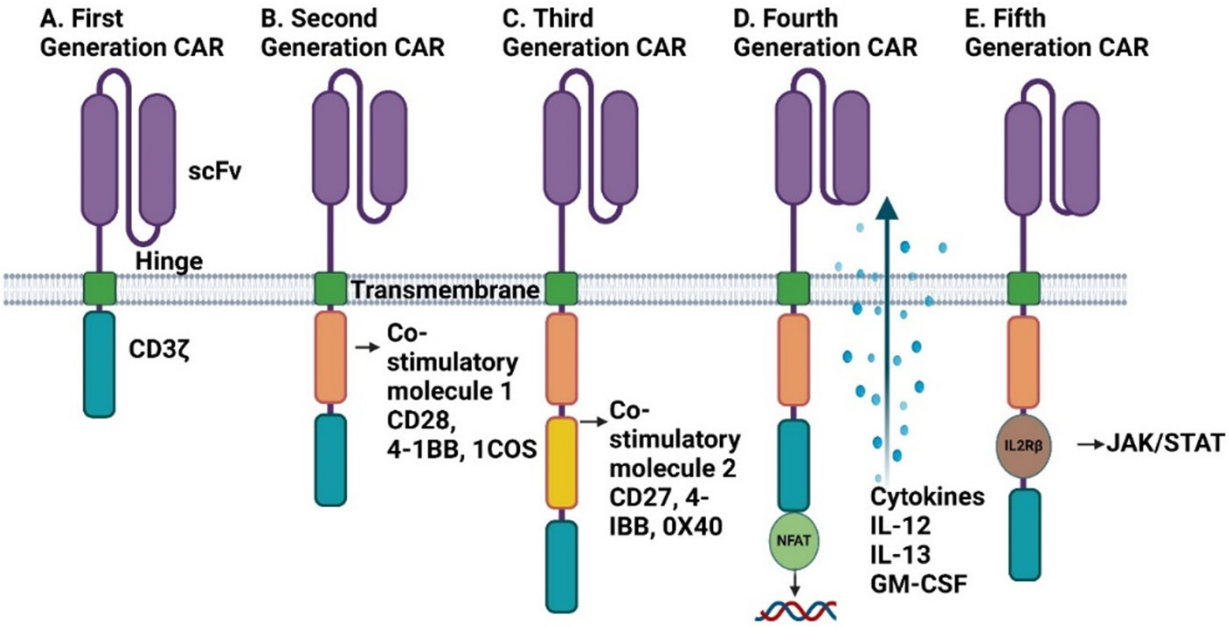

Figure 1: Evolution of Chimeric Antigen Receptors (CAR) from the first to the fifth generation. The first generation only has the CD3ζ signaling domain and is therefore not activated. The second generation has a co-stimulatory molecule with CD28, 4-1BB, or ICOS to fully activate the T-cell. The third generation has two co-stimulatory molecules, such as CD27, 4-1BB or OX40, for a stronger activation of the immune system. The inducible secretion of cytokines such as IL-12, IL-13, GM-CSF was used in the fourth generation, which also includes the "TRUCKs," to control the immune response within the tumor microenvironment. The fifth generation of CAR, which uses a new, mutated IL-2 receptor and the JAK/STAT pathway, offers even better therapeutic possibilities.

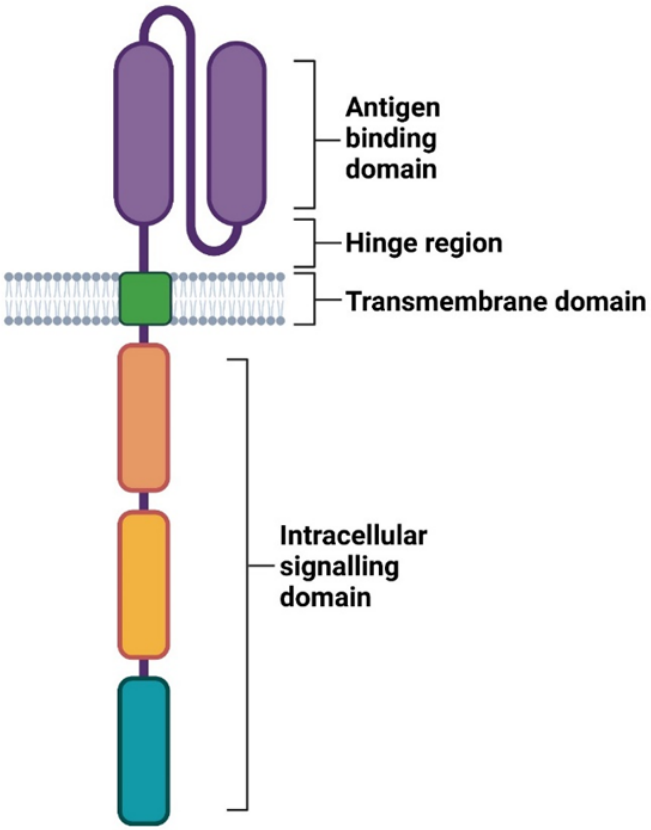

Figure 2: Structural Overview of the Chimeric Antigen Receptor (CAR) for improved cancer targeting. The extracellular antigen-binding domain, which consists of a single-chain variable fragment (scFv), is connected to a transmembrane segment by a flexible hinge. Within the cell, the signaling domain includes co-stimulatory molecules such as CD28 or 4-1BB and the CD3ζ chain, which promote T-cell activation and expansion, bypassing MHC restrictions.

Despite these promising advances, several challenges remain. The complexity of the TME with its dynamic and adaptive nature requires a deeper understanding of its interactions with DCLK1 and CSCs. Furthermore, improving the specificity and safety of therapies targeting DCLK1 is essential to minimize adverse effects while maximizing efficacy. Biomarker-driven approaches for patient selection, combined with advances in high-resolution molecular profiling, technologies will be critical for personalizing treatment regimens. In addition, addressing issues such as T-cell exhaustion, cytokine release syndrome, and antigen heterogeneity in solid tumors will require further innovation in T-cell engineering and combinatorial therapeutic strategies. The future of cancer treatment lies in the integration of DCLK1-targeted therapies with state-of-the-art immunotherapies, small molecule inhibitors, and precision medicine frameworks. These approaches hold the promise of not only improving survival rates, but also achieving durable remission and a better quality of life for patients by addressing the dual challenge of CSCs and the TME. The path to fully harnessing the potential of DCLK1-targeted strategies requires concerted efforts in translational research, clinical trials, and patient-centered care. Nonetheless, the advances outlined in this review provide a solid foundation for realizing the transformative potential of DCLK1 in the fight against gastric and other cancers (Figure 3).

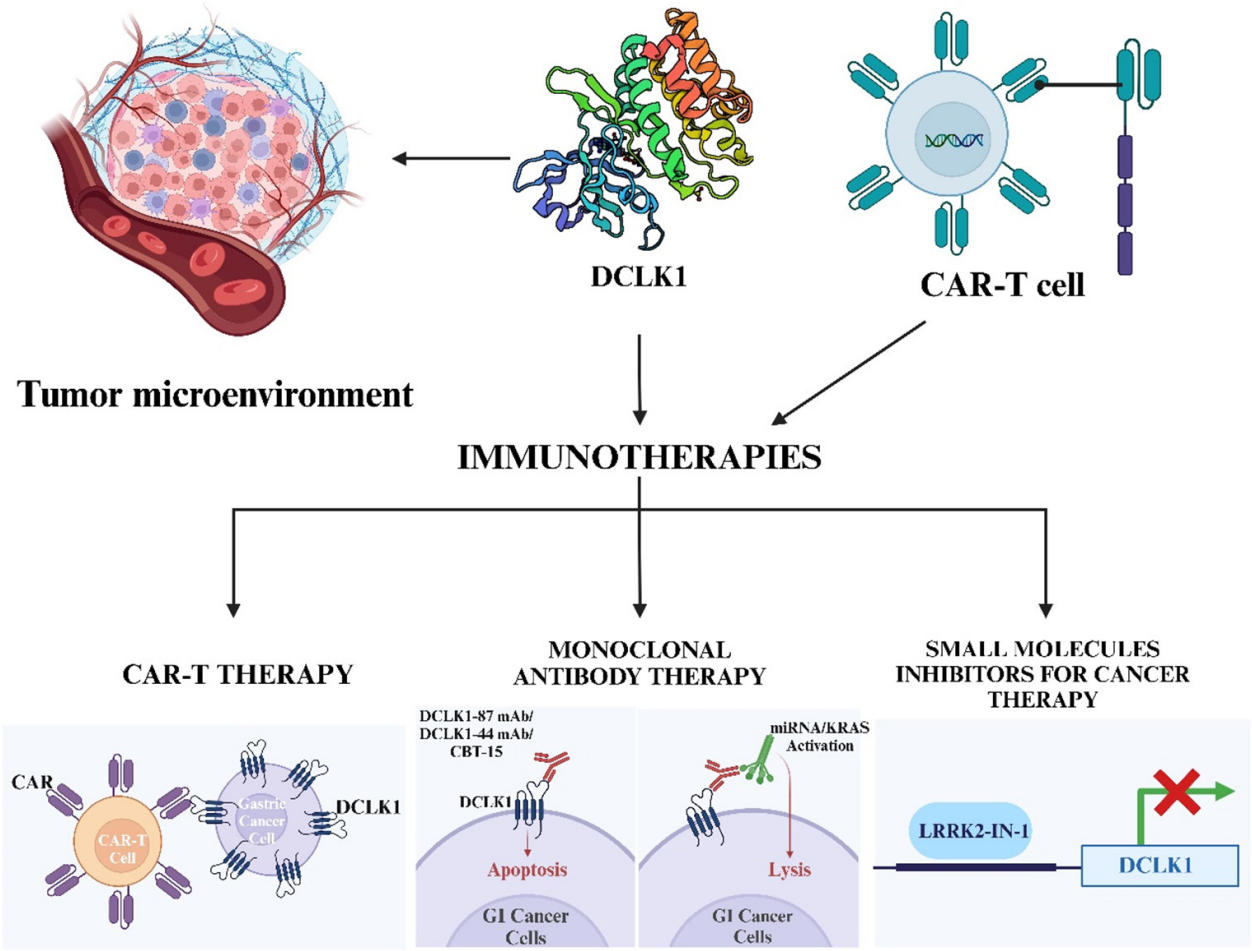

Figure 3: This graphical summary provides an overview of the potential of DCLK1 (doublecortin-like kinase 1) as a key target in cancer immunotherapy, particularly in gastric cancer. The tumor microenvironment is shown on the left, it shows how cancer cells interact with the surrounding stromal cells and blood vessels to promote tumor growth and resistance to therapy. In the center, DCLK1 is highlighted as a critical cancer stem cell marker, emphasizing its importance in cancer progression and its potential as a therapeutic target. Immunotherapy approaches are then categorized into three strategies: CAR-T Cell Therapy, in which specially engineered CAR-T cells are designed to recognize and attack DCLK1-expressing cancer cells; monoclonal antibody therapy, in which antibodies, such as DCLK1-87 and DCLK1-41, are used to induce cancer cell death while modulating key signaling pathways, such as miRNA/KRAS; and small molecule inhibitors, such as LRRK2-IN-1, block DCLK1 activity to halt tumor growth. Taken together, these approaches demonstrate the promising role of DCLK1 in developing targeted cancer therapies and overcoming the challenges posed by the tumor microenvironment.

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