

Review Article

Wnt signaling pathway protein LEF1 in cancer, as a biomarker for prognosis and a target for treatment

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Abstract: Transcription factors are regulatory proteins that either activate or repress the transcription of genes via binding to DNA regulatory sequences and regulating recruitment of transcriptional complexes. Lymphoid enhancer-binding factor 1 (LEF1), a member of the T-cell Factor (TCF)/LEF1 family of high-mobility group transcription factors, is a downstream mediator of the Wnt/ β -catenin signaling pathway, but can also modulate gene transcription independently. LEF1 is essential in stem cell maintenance and organ development, especially in its role in epithelial-mesenchymal transition (EMT) by activating the transcription of hallmark EMT effectors including N-Cadherin, Vimentin, and Snail. Aberrant expression of LEF1 is implicated in tumorigenesis and cancer cell proliferation, migration, and invasion. LEF1's activity in particular cancer cell types, such as chronic lymphocytic leukemia (CLL), Burkitt lymphoma (BL), acute lymphoblastic leukemia (ALL), oral squamous cell carcinoma (OSCC), and colorectal cancer (CRC), makes it a valuable biomarker in predicting patient prognosis. Additionally, due to aberrant LEF1 activity resulting in cancer progression, knockdown and inhibition treatments designed to target LEF1 have proven effective in alleviating cancer growth, migration, and invasion in CLL, CRC, glioblastoma multiforme (GBM), and renal cell carcinoma (RCC). In prostate cancer cells, LEF1 promotes androgen receptor expression and activity in an androgen-independent manner, ultimately increasing prostate cancer growth regardless of androgen ablation therapy. In this review, we review LEF1 regulation, its role in tumorigenesis in several cancer types, and its clinical value as a biomarker for predicting prognoses and as a target for treatment.

Keywords: LEF1, cancer, WNT, prostate, prognosis, treatment

Introduction

Lymphoid enhancer-binding factor 1 (LEF1) is a transcription factor that is primarily involved in the canonical Wnt/ β -catenin signaling pathway, and is implicated in tumorigenesis and progression of multiple neoplasms [1, 2]. Specifically, LEF1 is a facilitator of epithelial-mesenchymal transition (EMT), a hallmark of cancer cell migration and invasion, in addition to cancer cell proliferation and viability [3-6]. Unsurprisingly, prostate cancer is no exception to the effects of LEF1 as it modulates androgen receptor expression and activity independent of androgens, ultimately increasing prostate cancer growth potential and invasiveness, particularly in castration resistant prostate cancer (CRPC) [7]. LEF1's presence and dysregulation in a variety of cancer cell types makes it an

ideal biomarker predicting patient prognosis. LEF1's crucial role in propagating cancerous growth and metastasis also makes it an ideal target for treatment. Here we summarize LEF1's activity and regulation in both stem cells and cancer cells, with a primary focus on its role in prostate cancer. Additionally, we examine and discuss LEF1's particular role in cells undergoing EMT. Lastly, we discuss LEF1's potential as a biomarker for cancer detection and patient prognosis, as well as the utilization of treatment methods that target LEF1 to reduce cancer cell propagation.

LEF1 in WNT signaling pathway

The canonical Wnt/ β -catenin signaling pathway is a highly conserved developmental pathway involved in regulating cellular proliferation, dif-

ferentiation, organ development, tissue homeostasis and repair, and cellular apoptosis [1, 2]. Wnt binds to transmembrane Frizzled (Fz) proteins, which cooperate with lipoprotein receptor protein (LRP) family proteins to form a coreceptor complex. Wnt signaling results in the phosphorylation of the cytoplasmic segment of LRP, allowing Axin (axis inhibition protein) to dock [2]. Axin acts as a scaffold, in the absence of Wnt signaling, for the cytosolic β -catenin destruction complex, which includes β -catenin, adenomatous polyposis coli (APC), and two serine/threonine kinases: casein kinase 1 (CK1) and glycogen synthase kinase 3 β (GSK3 β) [8, 9]. When in this complex, β -catenin is phosphorylated at Ser45 by CK1, followed by phosphorylation at Ser33, Ser37, and Thr41 by GSK3 β , which is recognized and ubiquitinated by ubiquitinating proteins, and targeted for degradation by proteasomes [10]. Upon binding of Wnt to Fz/LRP, Axin is recruited to LRP by the plasma membrane, antagonizing the formation of the destruction complex, thus inhibiting the phosphorylation and degradation of β -catenin [1]. As a result, non-phosphorylated β -catenin translocates into the nucleus where it binds to the N terminus of LEF/TCF transcription factors and recruits co-factors to promote the transcription of Wnt target genes [2, 11, 12].

LEF1 belongs to the T cell Factor (TCF)/LEF family of transcription factors [13], containing a highly conserved high mobility group (HMG) DNA-binding domain and plays the role of nuclear effector in the Wnt/ β -catenin signaling pathway [12, 13]. In the absence of Wnt signaling, LEF1 is bound to Groucho-related co-repressors, thus negatively regulating the expression of Wnt signaling genes [14]. Upon stabilization from Wnt signals, β -catenin displaces the Groucho-related co-repressors and promotes LEF1 transcription factor activity [2].

LEF1 modulating EMT

EMT involves the generation of mesenchymal-like cells from epithelial cells. EMT is normally necessary for tissue repair processes and embryogenesis, especially during neural crest development [15]. During EMT, genes coding for cellular adhesion are inhibited, and in certain instances, cells produce proteolytic enzymes that degrade the extracellular matrix to enhance invasion and migration capacity

[15]. EMT is also associated with cancer recurrence and metastasis by increasing cellular motility, and poor survival in multiple cancers, including breast and colon cancers [16-18]. DLD1 colon carcinoma cells infected with adenovirus to overexpress LEF1 display EMT characteristics and activity, supporting the importance of LEF1 during EMT [19].

EMT markers include the upregulation of N-cadherin, Vimentin, and Snail, which are negatively correlated with E-cadherin, a calcium dependent transmembrane glycoprotein found in epithelial cells that mediates intercellular adhesion and cell polarity [18]. The intracellular domain of E-cadherin interacts with catenins (α -, β -, and γ -catenins) to form a link with the actin cytoskeleton [20]. In normal epithelial cells, β -catenin is tethered to E-cadherin. However, in instances of E-cadherin inhibition, free β -catenin localizes in the nucleus where it can bind and activate LEF1, increasing the transcription of oncogenes and increasing cell growth rate [20, 21]. During embryogenesis in palatal medial edge epithelial (MEE) cells, LEF1 represses the transcription of E-cadherin by recruiting phosphorylated Smad2 (Smad2-P) and Smad4, and directly interacts with the promoter region of the E-cadherin gene [22]. The E-cadherin promoter region contains two LEF1-binding regions (E-pal and non-E-pal) and a Smad binding element (SBE) for Smad4, where-in binding of the Smad2-P-Smad4-LEF1 complex at all three sites is necessary for E-cadherin suppression [22]. Decreased E-cadherin expression is observed in multiple prostate cancer cell lines, such as DU145, PC3, PPC1, and TSUPR1, and is indicative of metastasis [23].

N-cadherin, like E-cadherin, is a calcium dependent transmembrane glycoprotein, but is known for its involvement in cellular migration and neural tube development aside from being a biomarker for EMT [24]. In prostate cancer tissue samples, increased N-cadherin expression is associated with invasion, and shorter cancer recurrence and skeletal metastasis times [25]. In MCF7 cells, a weakly metastatic breast cancer cell line, overexpression of N-cadherin caused more efficient invasion and migration [26]. Suppression of E-cadherin and overexpression of N-cadherin occurs in an event called "cadherin switching", and is implicated in

prostate, ovarian, and breast cancer progression [25-28]. Inhibition of E-cadherin by LEF1 and persistence of N-cadherin allows cells to undergo EMT, thus promoting the migratory and invasive properties of cancer cells.

Vimentin, a type III intermediate filament protein, is found in mesenchymal cells and migratory epithelial cells [29]. The overexpression of Vimentin as a marker for EMT and metastasis is seen in multiple cancers [29-32]. LEF1 is implicated in the overexpression of Vimentin due to the activity of the β -catenin-TCF/LEF1 at the Vimentin promoter, found 468 bp upstream from the transcriptional start site [33]. Increased β -catenin-TCF/LEF1 expression and localization in the nucleus is implicated in migratory, Vimentin-expressing oral squamous cancer cells (OSCC) and breast cancer cells [18, 29].

The Snail super family, containing subfamilies Snail and Slug, are zinc-finger transcription factors that act as transcriptional repressors [34]. Snail and Slug are found to be implicated in breast cancer development [35-37]. Snail/Slug directly binds to the E-cadherin promoter and represses E-cadherin expression by recruiting histone deacetylase complexes, which remove acetyl groups from histones to compact chromatin [35-37]. In human osteoblasts, SLUG mRNA and protein expression increases due to LEF1 activity [38]. LEF1 regulates SLUG transcription by directly binding to TCF/LEF1 binding sites in the promoter, primarily at the site located at the -859/-855 position [38, 39]. IGF2 mRNA-binding protein 1 (IGF2BP1) promotes the expression of LEF1 by stabilizing LEF1 mRNA and preventing its degradation [5]. The increased expression of LEF1 promotes SNAI2 (Slug) expression and EMT processes [5].

LEF1 is also involved in the expression of micro RNAs (miRNAs) implicated in EMT. miRNAs are noncoding RNA molecules that specifically bind to the three prime untranslated region (3'-UTR) of target mRNAs, thus regulating post-transcriptional gene expression. In particular, the miR-181a family is induced by the Wnt/ β -catenin signaling pathway and is implicated in several cancers including lung, thyroid, and prostate cancer, and is associated with poor survival in patients with colorectal cancer [4, 40-43]. The β -catenin-TCF/LEF1 complex binds

to TCF/LEF1 binding sites (5'-A/T A/T CAAAG-3') located in the promoter region of miR-181a transcripts, indicating that the regulation of miR-181a is due to the activation of the Wnt/ β -catenin signaling pathway and direct binding of LEF1 [4, 41]. In prostate cancer and epithelial ovarian cancer (EOC) cells displaying greater miR-181a, N-cadherin and Vimentin expression, cell migration and invasion increases while E-cadherin expression decreases [41, 44]. In EOC, miR-181a regulates the TGF- β signaling pathway by increasing expression of mediators Smad2-P and Smad3-P, and decreasing expression of the inhibitor Smad7 [44]. In hepatocellular carcinoma cells (HCC), miR-181 maintains stem cell attributes by targeting an inhibitor of the Wnt/ β -catenin signaling pathway, nemo-like kinase (NLK), and the regulatory transcription factors involved in differentiation, caudal type homeobox transcription factor 2 (CDX2) and GATA binding protein 6 (GATA6) [45].

LEF1 does not require β -catenin recruitment in order to elicit EMT. Expression of LEF1 and Δ NLEF1, a mutant form of LEF1 lacking the β -catenin binding site, increase EMT potential and expression of EMT markers, such as Slug and ZEB1, in Madin-Darbin canine kidney (MDCK) cells [6]. Inhibition of β -catenin transcription and activity does not alleviate the effects of LEF1-induced EMT [6]. During mouse palate development, β -catenin is not located in the palatal nuclei before, during, or after EMT [46]. These studies indicate that LEF1 regulates EMT in normal, early development and also in cancer cell invasion and migration. LEF1 regulates EMT markers, and unregulated LEF1 expression can result in increased EMT qualities. While the Wnt/ β -catenin signaling pathway is a possible course for EMT, it is not the only pathway nor is it necessary for LEF1-induced EMT activity.

Regulation of LEF1 expression

LEF1 has a central role as a Wnt/ β -catenin signaling pathway mediator and downstream cellular effects, making it a key regulatory factor for eliciting or preventing aberrant protein expression. ETS family transcription factor ERG protein is an activator of the Wnt/ β -catenin signaling pathway and upstream regulator of LEF1 expression. Approximately 50% of human prostate cancers contain chromosomal

rearrangements induced by androgen or genomic stress, that place the TMPRSS2 promoter next to the coding region for ERG, resulting in increased ERG expression [47-50]. ERG expression regulates Wnt ligand expression in prostate cancer cells and increases active β -catenin without altering total β -catenin level [51]. Aside from regulating LEF1 through Wnt signaling and β -catenin binding, ERG also regulates LEF1 transcription by binding to sites located in the LEF1 promoter [51]. In ERG-overexpressing prostate cancer cell lines (LNCaP, 22Rv1, and DU145), LEF1 expression is greatly induced while other members of the TCF family do not experience altered gene expression, thus indicating the importance of LEF1 in eliciting ERG's effects in prostate cancer [51].

Natural antisense transcripts (NATs) are non-coding RNA that are transcribed in the opposite direction to the mRNA of the coding gene [52]. NAT levels alter the expression of their gene counterparts and can silence target genes [53-56]. The unspliced version of the LEF1 NAT inhibits LEF1 transcription by binding to the LEF1 promoter and inhibiting its activity [57]. Meanwhile, the spliced version of the LEF1 NAT inhibits the activity of the unspliced version [57]. Additionally, LEF1 NATs induce LEF1 promoter repression by promoting histone modification via facilitation of recruitment of the PRC2 complex [57].

LEF1 is also regulated by miRNAs. miR-218 has been implicated in the suppression of cancer progression [58]. Specifically, miR-218 regulates and suppresses LEF1 expression by binding to the 3'UTR of LEF1 mRNA, limiting the invasiveness of glioblastoma cells [59]. miR-34a also has a tumor suppressor role and suppresses prostate cancer progression and metastasis, and promotes apoptosis [60-62]. Similar to the activity of miR-218, miR-34a negatively regulates the expression of LEF1, ultimately diminishing the EMT capability of prostate cancer cells [63, 64]. miR-223 and miR-34a downregulate LEF1 expression and allow for proper macrophage differentiation [65].

Variable expression of specific LEF1 isoforms formed by alternative splicing have been observed to possess differential transcriptional activity. The truncated LEF1 isoform lacks

the first 113 amino acids due to a second promoter located in intron 2, subsequently removing the β -catenin binding site [66, 67]. While the full length LEF1 isoform promotes cell proliferation via Wnt/ β -catenin signaling, the truncated isoform is thought to be inhibitory with its ability to compete for DNA binding sites [66-68]. Increased expression of truncated LEF1 in colon cancer cells results in reduced growth, colony formation, migration, and adhesion, defective angiogenesis, and increased apoptosis via caspase-3 activity [69].

The cancer types mentioned thus far involve the aberrant expression and activity of LEF1. LEF1 dysregulation and oncogenic potential has been linked to the suppression or excitation across several different regulatory mechanisms. These studies elucidate the importance for appropriate LEF1 regulation in order to maintain benign cell functioning.

LEF1 function in stem cells

LEF1 plays a critical role in stem cell maintenance via the Wnt/ β -catenin pathway. Only a small percentage of LEF1 deficient fetal mouse liver pro-B cells enter the cell cycle relative to wild type pro-B cells [70]. LEF1 is expressed in mouse fetal liver cells, while it is not significantly expressed in adult spleen and bone marrow cells, supporting LEF1's role in early B-cell differentiation. Additionally, LEF1^{-/-} cells display a 20-fold increase in apoptosis relative to wild type cells through the increased expression of fas and c-myc genes, indicating the deregulation of fas and c-myc in the absence of LEF1 [70]. LEF1^{-/-} fetal liver pro-B cells also display 50% decrease in DNA synthesis and a 50% decrease of cells in S, G2, and M phase of the cell cycle, indicating LEF1's significance in cell proliferation, cell cycle progression, and early stage development.

Mesenchymal stem cells (MSC) are multipotent stem cells that are capable of multilineage differentiation, including osteoblasts, chondrocytes, adipocytes, and myoblasts [71-73]. Wnt/ β -catenin signaling is involved in suppressing differentiation in MSCs; MSCs grown in Wnt3a-conditioned media displayed suppressed levels of mRNA for bone sialoprotein and alkaline phosphatase, markers for differentiation [74]. In addition to preventing differentiation,

MSCs grown in Wnt3a-conditioned media displayed increased proliferation and decreased apoptosis [74]. Human adipose stromal cells, a type of human MSC, grown in Wnt3a-conditioned media increased cellular β -catenin levels, thus increasing cellular proliferation and inhibiting osteogenic differentiation [75]. Overexpression of Wnt7a promotes proliferation and self renewal in neural stem cells in the presence of epidermal growth factor (EGF) and fibroblast growth factor (FGF) [76]. Similar patterns of proliferation, self-renewal, and reduced differentiation via Wnt/ β -catenin signaling and LEF1 activity are also observed in hematopoietic stem cells, human keratinocytes, and intestinal stem cells [77-80].

Hematopoietic (HSC) and leukemic stem cells (LSC) display a dependence on LEF1 in order to maintain stem cell qualities. LEF1^{-/-} and Tcf7^{-/-} LEF1^{-/-} bone marrow cells show a reduction in their competitive repopulation potential [81]. Tcf7^{-/-} LEF1^{-/-} HSC show a reduction of population in the G0 phase and increase in the G1 phase and entering into the cell cycle, indicating TCF7 and LEF1's role in maintaining HSC quiescence [81]. Tcf7^{-/-} LEF1^{-/-} LSC transplanted into secondary host mice give rise to less leukemic cells compared to wild type LSC, suggesting LSC dependence on TCF7 and LEF1 for self-renewal [81].

Some key genes that confer stem cell qualities include c-myc, cyclin D1, Oct4, and nanog, and siRNA knockdown of these gene products initiates differentiation [82-84]. These genes are also downstream effectors of Wnt signaling, and are activated by the recruitment of LEF1 to their respective promoter sites. C-myc is a transcription factor that is essential for embryonic development and regulates the transcription of genes involved in the cell cycle, and targets molecules involved in the G1/S transition such as CDK2, CDK4, CDC25A, and E2Fs [85]. Cyclin D1 is involved in cell cycle progression, especially in the G1 phase, and is necessary for growth and proliferation [86]. The promoters for c-myc and cyclin D1 contain LEF1 consensus sequences that allow β -catenin-LEF1 to bind and modulate c-myc and cyclin D1 expression [87-89].

Oct4 and nanog are transcription factors associated with regulating pluripotency in stem cells at appropriate expression levels [90-92]. The Oct4 promoter contains 3 putative TCF/LEF1

binding sites, from -1853 to -1846, -1642 to -1635, and -847 to -840 [93]. Furthermore, LEF1 binds to the site located from -847 to -840 with high specificity and induces Oct4 promoter activity. The nanog promoter contains two TCF/LEF1 binding sites at -1206 to -1190 and -1020 to -1004, and β -catenin-LEF1 activity in conjunction with Oct4/sox2 induces nanog promoter activity [94]. Overexpression of LEF1 increases the expression of Oct4 and nanog [93]. In addition to promoting nanog expression, LEF1 binds with nanog to enhance transcriptional activity [93]. Activation of the Wnt signaling pathway and LEF1 maintains undifferentiated embryonic stem cell morphology [93, 95]. Inhibition of the Wnt signaling pathway and reduced LEF1 expression causes a flattened morphology, reduced expression of Oct4 and nanog, and differentiation of embryonic stem cells [93, 95].

It should be noted that there are also certain instances wherein LEF1 expression facilitates stem cell differentiation. Hair follicle stem cells differentiate after receiving signals from dermal papilla [96]. Dermal papilla induction elevates the expression of LEF1 and its downstream effectors β -catenin and c-myc, ultimately determining hair fate [97]. LEF1 is also implicated in the maturation of natural killer T cells and T follicular helper cells [98-100]. LEF1 regulates Cd127 and c-myc in natural killer T cells, and regulates multiple stages in invariant natural killer T cell proliferation, survival, and differentiation [98]. Inhibition of LEF1 diminishes T follicular helper cell differentiation, while increased LEF1 expression enhances differentiation by increasing IL-6 expression, an early signal that commands differentiation [50].

LEF1 in carcinogenesis

Altered LEF1 expression and function commonly occur in several cancers, such as lung adenocarcinoma, colon cancer, endometrial carcinoma, prostate cancer and leukemia [7, 12, 101-104]. Several of the target genes of the Wnt/ β -catenin-LEF1 pathway that are responsible for conferring stem cell properties are also the same genes that promote cancer development.

Wnt pathway target genes, including but not limited to c-Myc, LBH, Oct4, nanog, and LEF1 have been associated with the upregulation of

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proteins typically involved in human breast cancer, gastrointestinal tumors, prostate cancer, leukemia, and others [66, 87, 88, 93, 94, 105-111]. Additionally, the Wnt pathway and LEF1 have been associated with EMT, a hallmark of carcinoma progression and metastasis, wherein epithelial-like tumor cells generate mesenchymal-like characteristics, including decreased cell-cell adhesion and increased migration, invasion, and apoptosis-resistant properties [3-6].

Breast cancer is the most common cancer in women and the third leading cause of cancer related deaths for the whole population in the United States [112]. Phthalates, which are a group of environmental hormones found in cosmetics, toys, medical equipment, and other plastics, disrupt endocrine function when consumed or come into contact with skin [113, 114]. N-butyl benzyl phthalate (BBP) has been proven to increase the viability, proliferative, invasive, and migration abilities of breast cancer cells and induces the expression of oncogenes such as HDAC6 and c-myc [115-118]. However, BBP effects require LEF1. Suppression of LEF1 reduces BBP's effectiveness in activating cell growth, invasion, and migration [118]. Hepatocyte growth factor (HGF) is a secretory cytokine capable of inducing cell proliferation, invasion, migration, survival, and angiogenesis in cancer cells [119, 120]. HGF increases LEF1 expression by activating the Akt/NF- κ B pathway, and utilizes LEF1 to induce tumor invasion and migration, especially in breast (MDA-MB-231) and liver cancer (HepG2) cell lines [121].

Lung cancer is the leading cause of cancer-related deaths in the United States [112]. Overexpression of LEF1 and HOXB9 in lung cancer cells increases the metastatic activity to bone and brain regions [104]. Specifically, increased LEF1 and HOXB9 expression observed in lung cancer cells potentiate increased chemotactic invasion and outgrowth [104].

LEF1 is not expressed in normal adult colon tissue, however it is activated during colon carcinogenesis [101]. β -catenin, along with TCF1 or TCF4, can activate the full-length LEF1 promoter and stimulate LEF1 expression [66]. In addition to increased β -catenin activity in colon cancer tissue, there is reduced expression of

the truncated LEF1 isoform, which suppresses the activation of downstream target genes [66]. Due to a promoter site located in the second intron, transcription begins at exon 3, resulting in a truncated LEF1 protein missing a β -catenin binding domain [66]. The truncated isoform's inability to bind to β -catenin, but with a functional DNA binding site, allows it to compete for binding sites thus preventing β -catenin recruitment. These characteristics of the truncated LEF1 make it a natural antagonist to full-length LEF1. However, in colon cancer development and progression, there is an inappropriate activation of the full-length LEF1 protein by β -catenin, and the promoters for both the full-length and the truncated isoforms of LEF1 are differentially regulated.

In murine models, LEF1 is involved in uterine development and gland-bud formation [102]. LEF1 protein expression returns to baseline levels when the majority of gland formation is complete [101]. During the estrus cycle LEF1 levels elevate, correlating with cellular proliferation and gland formation in proestrus [102]. While LEF1 is present and necessary in normal endometrial development and estrus cycling, it is overexpressed in human endometrial cancers and is primarily located in glandular portions of the tumors [101]. Downstream targets of LEF1 and markers for endometrial cancer, cyclin D1 and MMP7, demonstrated a 10-fold and a 30-fold increase in RNA, respectively, in high-LEF1 expression endometrial tumors compared to inactive endometrial tissues [102].

LEF1 is necessary in T- and B-cell differentiation and proliferation, and thus is normally expressed in human bone marrow [12, 39, 122]. However, there is an approximate 13-fold increased expression level of LEF1 in acute lymphoblastic leukemia [12]. Overexpression of LEF1 induces acute myeloid leukemia in murine models [12]. Adult acute lymphoblastic leukemia patients expressing high levels of LEF1 mRNA display higher median white blood cell counts and a higher percentage of lymphoblasts in blood than patients expressing low levels of LEF1 mRNA [103]. Point mutations located in exons 2 (K86E) and 3 (P106L) of LEF1 result in increased promoter activity and expression for c-myc and cyclin D1, causing increased leukemia cell proliferation [103].

These studies demonstrate the importance of LEF1 in elucidating typical cancer characteristics, including proliferation, invasion, migration, and viability, amongst a variety of cancer types, and highlight its necessity in propagating these effects. Not only is LEF1 at the center of signaling pathways and mechanisms that initiate and maintain carcinogenesis, suppression of LEF1 reduces the proliferative and invasive properties of cancer. These findings demonstrate that LEF1 plays a crucial role in cancer survival and activity.

Prostate cancer is the most common cancer amongst U.S. men and is one of the leading causes of cancer-related deaths [112]. Prostate gland function is primarily driven by the androgen receptor, and androgen ablation therapy resolves prostate cancer progression initially. However, a majority of prostate cancers develop into the more lethal hormone-refractory prostate cancer or CRPC after patients undergo hormonal therapies targeting the reduction of androgen [123, 124]. Despite the deficit levels of circulating androgen, human androgen receptor protein expression and androgen mediated gene expression is still sustained [125]. Altered function via mutation or the overexpression of androgen receptor plays a major role in the development and metastatic potential of prostate cancer [126-130]. The Wnt/ β -catenin pathway is also implicated in both androgen dependent and androgen independent prostate cancer progression through modulation of androgen receptor's transcriptional activity, even in the absence of androgens [131-133]. β -catenin binds to androgen receptor and acts as a coactivator, augmenting androgen receptor's transcriptional activity and specificity to androgens and antiandrogens [134, 135].

Downstream from the Wnt/ β -catenin pathway, LEF1 plays a role in prostate cancer development, especially in androgen independent phenotypes [7]. A 100 fold increase in the expression of LEF1 and higher co-localization with androgen receptor in the nucleus are observed in LNCaP-AI cells (an androgen-independent LNCaP derivative) when compared to LNCaP cells [7]. LEF1 binds to sites located in the human androgen receptor promoter site, thus inducing greater expression of human androgen receptor in PC3, DU145, and LNCaP human

prostate cancer cell lines [7, 136]. LNCaP-AI cells with increased expression of androgen receptor display a greater potential for invasion and growth than LNCaP [7]. To determine whether LEF1 drives increased growth and invasion observed in LNCaP-AI cells, LNCaP cells, which normally display lower levels of LEF1 expression, were transfected with a vector containing LEF1 to induce increased LEF1 expression. The expression level of LEF1 in the LNCaP-LEF1 cells was comparable to expression levels observed in LNCaP-AI. LNCaP-LEF1 cell populations displayed a 30% increase in S-phase cells and a 2.5 fold increase in invasive ability by Matrigel assay [7]. To mimic decreased LEF1 expression comparable to LNCaP cells, LNCaP-AI cells were treated with LEF1 shRNA to stably knockdown LEF1 expression. These LNCaP-AI cells displayed a significant reduction in S-phase cells and a 3 fold decrease in invasion ability [7]. These experiments indicate the importance of LEF1 in modulating androgen receptor, and subsequently, prostate cancer growth and invasion capabilities.

Similar to other cancer types mentioned earlier, increased LEF1 is implicated in EMT in prostate cancer cell lines. Increased ERG expression in conjunction with increased LEF1 expression causes EMT [74]. LEF1 has also been observed to increase the expression of miRNAs associated with prostate cancer EMT. LNCaP-AI cells experience a 12.36-fold increase in miR-181a due to increased LEF1 expression and activity at the miR-181a promoter region compared to its androgen dependent counterpart, indicating a positive correlation between LEF1 and miR-181a expression [4]. Increased N-cadherin and decreased E-cadherin, hallmarks for EMT, and increased migration and invasion capabilities were observed with miR-181a overexpression in LNCaP cells. As mentioned before, miR-181a is implicated in EMT in numerous cancer types, including prostate cancer, by regulating EMT gene expression.

LEF1 as a biomarker for cancer prognosis

LEF1 has been indicated as a useful diagnostic marker for development, metastases, and poor prognoses in several cancers. The ability to diagnose using LEF1 provides the ability to identify the presence, type, and prognosis of

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specific cancers with more certainty. Ultimately, LEF1's important characteristics identify it as a potential target for specialized therapies and novel therapeutic agents designed to effectively treat patients.

Chronic lymphocytic leukemia (CLL) is a B lymphocyte hematological malignancy, marked by proliferation and accumulation of B cells in blood, bone marrow, and lymphoid tissues [137, 138] and currently has no effective treatment. Primary diagnosis for CLL has been based on morphological evaluation and immunophenotyping and LEF1 has been indicated as an effective marker in CLL [139]. LEF1 expression is normally inactivated in mature B cells, however LEF1 overexpression is observed in CLL compared to normal B cells [140-144]. Tanden et al. showed 100% of their 92 CLL neoplastic samples express nuclear LEF1 strongly, and Menter et al. also indicated that 70% of chronic lymphocytic B cell leukemia cases express LEF1 [142, 143]. Amongst CLL cases, patients exhibiting higher expression levels of LEF1 have poorer prognoses with lower overall survival times compared to patients with low LEF1 expression [144]. In addition to strong LEF1 expression in CLL cases, Tanden et al. add that only 38% of their 71 diffuse large B cell lymphoma (DLBCL) cases express LEF1 [142]. This differential expression of LEF1 could provide a method to differentially diagnose between morphologically indistinguishable types of B cell lymphomas. Additionally, the lack of nuclear β -catenin activation and localization further posits the importance and necessity of using LEF1 as a biomarker for diagnosing cancer [142, 143].

Burkitt lymphoma (BL) is an aggressive and malignant form of non-Hodgkin's lymphoma that arises from germinal center B cells. Walther et al. showed that 83% of their 18 (BL) cases express nuclear LEF1 while normal germinal center B cells do not express nuclear LEF1 [145]. Furthermore, similar to CLL, LEF1 in BL cases is overexpressed compared to DLBCL cases, making LEF1 a valuable marker in differentiating lymphoma types [145].

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor in the head and neck region [146]. Prognostic factors utilized in clinical practice are unable to differentiate tumors at the same clinical stage [147] and cur-

rent 5-year survival rates are approximately 50% [148, 149]. LEF1 is overexpressed in OSCC compared to non-tumorous oral mucosa while TCF4, another transcription factor in Wnt/ β -catenin signaling, is not differentially expressed between cancerous and noncancerous cells [146]. Higher LEF1 expression is associated with lymphovascular invasion and reduced overall survival [146]. The fact that LEF1 is differentially expressed between normal and cancer tissue while other marker expression, such as TCF4, remains constant supports LEF1 as an ideal biological marker for distinguishing OSCC from non-tumorous oral mucosa. Additionally, LEF1 expression is significantly associated with poor prognosis, making it an excellent marker for predicting patient outcome and appropriate treatments.

LEF1 expression is significantly greater in colorectal cancer (CRC) tissues than in paratumorous normal colorectal cells, which is negatively correlated with Notch2 expression [150]. High LEF1 and low Notch2 expression patterns are associated with tumorigenesis, shorter overall survival time, and higher risk of death in CRC patients [150, 151]. Additionally, the presence of increased LEF1 is associated with an increased risk for colorectal liver metastasis [151]. With colon cancer being responsible for approximately 51,000 cancer related deaths every year, using LEF1 as a biomarker will be valuable to identify and improve prognosis of CRC [80].

Within cerebrally metastasized lung adenocarcinoma patients, a subgroup of patients expressing higher levels of nuclear LEF1 and TCF4 exhibited poor prognosis with significantly shorter survival times [152]. Similar to CLL studies, nuclear β -catenin had no significant effect on the prognosis of patients, further demonstrating LEF1 as an effective biomarker for specific cancer types [152].

While high LEF1 expression has been attributed to negative prognoses, overexpression of LEF1 has been identified to be a positive prognostic factor in pediatric acute lymphoblastic leukemia (ALL). ALL is the most common malignancy amongst children, in which 80% of cases are curable with current methods [153]. However, 20% of patients experience relapse, wherein several cases are originally considered low risk [154]. The ability to correctly diagnose

the severity of the patient's malignancy would allow for an appropriate response and therapy in treating high risk ALL. Patients with high expression of LEF1 had lower pretreatment white blood cell counts and minimal residual disease than patients with low LEF1 expression [155]. Additionally, patients expressing high LEF1 levels displayed higher complete remission rates and longer overall survival and relapse-free survival times [155].

LEF1 as potential therapeutic target

LEF1's central role as a transcription factor in the Wnt/ β -catenin signaling pathway and its additional activity independent of β -catenin binding make it an ideal target for therapeutic treatment in dealing with cancer proliferation. Knockdown of LEF1 in colon cancer cells causes increased apoptosis compared to control cells *in vitro*, and reduced tumor growth compared to normal colon cancer cells *in vivo* [156]. Additionally, the knockdown of LEF1 reduces colon cancer cells (SW480 and SW620) invasiveness via decreased MMP-2 and MMP-9 expression [156]. Selenite treatment of colorectal cancer cells triggers apoptosis by inhibiting LEF1 regulatory activity [157]. Caspase-8 is activated through the deubiquitination of receptor-interacting protein 1 (RIP1) by cylindromatosis (CYLD) [158]. However, LEF1 negatively regulates CYLD by binding to the CYLD promoter site, repressing its transcription [159]. Rather than decreasing LEF1 expression, selenite treatment inhibits LEF1 recruitment to the CYLD promoter site [157]. Average colorectal cancer tumor weight is significantly less in selenite treatment groups compared to the negative control [157]. Additionally, TUNEL assays indicate that selenite treatment causes increased DNA fragmentation, a marker for apoptosis [157].

Glioblastoma multiforme (GBM) is the most malignant, aggressive, and common form of brain cancer, with GBM patients' mean survival being only 14.6 months [160]. LEF1 knockdown in U251 GBM cells inhibits invasion, migration, proliferation, and the self-renewal potential of stem-like cells [161]. It has been observed that miR-218 is downregulated in GBM compared to normal brain tissue, and this pattern is also seen in cervical cancer and lung cancer [127, 162, 163]. miR-218 is inversely related to MMP-7 and MMP-9 expression in

GBM tissues, both of which are effectors of the Wnt signaling pathway [127, 164]. miR-218 regulates MMP-9 expression by directly targeting the 3'UTR of LEF1 mRNA, thus downregulating LEF1 expression [127]. LN229 glioblastoma cells transfected with miR-218 display reduced invasion and migration potential by approximately 3-fold and 2-fold, respectively, compared to negative controls [127]. Using miR-218 as a treatment due to its ability to inhibit LEF1 expression and activity reduces GBM cells' metastatic potential.

Ethacrynic acid (EA) is a loop diuretic drug that is cytotoxic to several cancer cell lines, especially primary CLL cells, myeloid leukemia cells, and human colon cancer cells [165-168]. Primary CLL cells are highly sensitive to EA compared to normal peripheral blood cells, making it a selective drug [144, 165, 168]. EA inhibits the Wnt/ β -catenin signaling pathway by reducing expression of Wnt target genes, including LEF1, and destabilizing the β -catenin/LEF1 complex by directly binding to LEF1 [168]. Furthermore, EA reduces LEF1's capability to bind to DNA [144]. EA's interference in LEF1 expression and activity as a transcriptional repressor allows for increased CYLD expression and necroptosis, the programmed necrosis induced by stimulation of death receptors [144, 169]. Similar to observations with CYLD in colorectal cancer, increased CYLD expression and activity promotes CLL apoptosis [144].

5-aza-2'-deoxycytidine (DAC) is a DNA methyltransferase inhibitor used in the treatment of several cancer types [170, 171]. In combination with DAC, paclitaxel (PTX), a chemotherapeutic agent, inhibits the growth of renal cell carcinoma (RCC) [172]. PTX and DAC synergistically decrease LEF1 expression *in vivo*, resulting in decreased RCC proliferation [173]. Additionally, DAC increases the sensitivity of RCC to PTX, and increased synergistic effects are found in RCC cells with higher LEF1 expression levels than in normal cells [173].

Conclusion

The specificity of aberrant LEF1 expression and activity, and its numerous, malevolent downstream effects, makes it an effective and accurate marker for tumor diagnosis and prognosis. The general trend for using LEF1 as a biomark-

er has shown that increased LEF1 expression results in poorer prognoses, therefore demanding more aggressive therapy [144, 146, 150-152]. However, there are several cases wherein high LEF1 expression is a hallmark for favorable outcomes, indicating the effectiveness of current treatments [155]. Future research is necessary to evaluate the effectiveness of using LEF1 as a biomarker for other cancer types and for providing an objective standard for evaluating the severity of the patient's condition.

In addition to using LEF1 as a biomarker, LEF1 has the potential to be a potent direct cancer therapeutic target, however further research is still needed. While other treatments can target various aspects of the Wnt/ β -catenin signaling pathway, LEF1 is unique in that it has also been shown to function independent of β -catenin activity [6, 142-145]. Targeting LEF1 would provide a more direct method in preventing cancer proliferation and metastasis. In vivo experiments indicate the effectiveness of reducing the invasion, migration, proliferation, and viability capabilities of cancer cells by reducing LEF1 expression [20, 89, 156, 157, 161]. Medications including selenite, EA, DAC, and PTX are proven effective cancer drugs due to their ability to limit LEF1 expression [144, 157, 168, 173]. Additionally, the usage of NATs and miRNAs inhibit LEF1 expression, providing another avenue for researching possible treatment options [125, 132, 133].

Lastly, further research in the differential transcription and expression of LEF1 isoforms would provide insight in maintaining appropriate LEF1 levels. Specifically, the preferences for differential expression should be explored in order to understand the delicate balance between LEF1 and its truncated isoform, which is able to compete for DNA binding sites and act as a repressor for Wnt/ β -catenin genes [66]. In-depth understanding of LEF1 function and clinical usefulness will provide more effective treatments in inhibiting or reducing cancer progression by maintaining appropriate LEF1 expression and activity.

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Disclosure of conflict of interest

None.

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