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Potential involvement of mi-RNA 574-3p in progression of prostate cancer: A bioinformatic study

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ABSTRACT

Aberrant gene expression is a hallmark of prostate cancer (PCa), the second deadliest disease affecting males worldwide. Dysregulation of miRNA has been associated with the progression of PCa and in recent studies, miRNA 574-3p was found to be upregulated in cancerous prostate tissue. In this study, we characterize the effects of upregulated miRNA 574-3p on gene expression in the tumor microenvironment through different bioinformatic tools such as Diana-Tools, the KEGG Pathway Database, and the Reactome Database. We have identified nine regulatory genes that are targeted by miRNA 574-3p and downregulated in prostate cells. Pathway analysis of these genes shows that they are involved in the regulation of the Notch signaling pathway, Wnt signaling pathway, apoptosis, DNA damage response, G1 to S cell-cycle control, inflammatory response pathway, angiogenesis, translation factors, and the expression of oncogenes. Our results show the oncogenic potential of miRNA 574-3p in PCa progression and metastasis. Moreover, this study highlights the complex molecular mechanisms and pathways affected by the upregulation of miRNA 574-3p in prostate cells. In future studies, the presented data may aid in designing new therapies for PCa with improved efficacy.

1. Introduction

Prostate cancer (PCa) is the second leading cause of mortality in men. Each year, approximately 1.1 million men are diagnosed with PCa worldwide [1]. Among many causative factors of PCa, aberrant gene expression has been shown to be most significant and has been linked to overexpression or suppression of microRNAs (miRNA) [2,3]. Under such conditions, miRNAs silence gene expression by targeting the complementary mRNA sequence more frequently in the 3′ untranslated region, thus inhibiting protein synthesis [4]. Several miRNAs are found to be upregulated in cancerous prostate cells and result in the alteration of cell signaling cascades that are either directly or indirectly involved in metastasis [5].

In the last decade, some miRNA expression studies involving PCa have been conducted to evaluate the fluctuating levels of miRNA in PCa patients compared to healthy individuals. Notably, miRNA 574-3p was found to be upregulated in the plasma and serum of PCa patients, which was associated with changes in the cellular environment such as genotoxic stress and dysregulation of the tumor suppressor genes [6].

Interestingly, miRNA 574-3p has also been identified in membrane-bound vesicles called exosomes, which increase miRNA stability through RNase evasion [7]. As a result, they are easily distributed to neighboring cells through endocytosis and may alter gene expression in nearby cells, leading to abnormal cell proliferation [8].

Furthermore, given the laborious process required to validate the wide range of target sites of a single miRNA, this study seeks to provide insight into the cancerous behavior of PCa through the identification of PCa correlated genes targeted by miRNA574-3p [4]. This study emphasizes a pathway oriented approach to identify potential mediators involved in cancer metastasis and development. Moreover, this study makes progress toward the eventual validation of several novel signaling pathways involved in PCa.

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2. Methodology

2.1. Selection of miRNA

miRNA 574-3p was used as a key query due to its upregulation in the serum of PCa patients as described above. Moreover, its cargo has been observed in the exosome and is released by cancerous prostate cells. On this basis, miRNA 574-3p may affect the normal expression of genes in nearby cells, resulting in cancerous behavior [9]. This miRNA is further subjected to a bioinformatic pipeline to identify its potential targets (Fig. 1).

2.2. DIANA Tools

DIANA TOOLS (diana.imis.athena-innovation.gr/) was used to predict miRNA 574-3p target genes in cells and was used in conjunction with DIANA-microT-CDS (http://www.microrna.gr/microT-CDS) to enhance predictive power by leveraging its human specificity and its machine learning approach [10]. Furthermore, this program is designed specifically for the positive and negative set of miRNA recognition elements (MREs) on both the 3′ UTR and CDS regions. The output consists of two separate scores as well as a common score called the miTG score. The threshold was set to 0.7 to rule out false positive and negative results.

2.3. Sorting and selection of targeted genes

Genes identified by microT-CDS Tools were then further sorted by their involvement in carcinogenesis through a comprehensive literature review and bioinformatic mining of the Human Protein Atlas Database (http://www.proteinatlas.org/) [11].

The literature review was carried out to exclude genes which were not involved in cancer development and metastasis. Genes selected by DIANA Tools have been rigorously filtered by optimizing key parameters during literature review. Only those genes which the literature has shown to be directly involved in development, metastasis, and the metabolic pathways of cancer were used in this study. To further shortlist genes of interest, The Cancer Atlas (http://www.proteinatlas.org/cancer) was used to isolate genes which were expressed in PCa, and all others were neglected.

2.4. Pathway design

A comprehensive pathway for each of the selected genes was retrieved using the databases mentioned above or compiled when not available. Various pathway databases, such as KEGG and Reactome, and available literature were also used to identify genes relevant to PCa. A summary of each is provided below.

2.4.1. KEGG

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a publicly available integrated database (http://www.genome.jp/kegg/) used primarily in the interpretation of high-throughput data, the interpretation of high-level functions, and the identification of possible utilities in biological systems [12]. The KEGG Pathway Database contains graphical information of biochemical and regulatory pathways that decipher the complex pathways through which genes and molecules interact.

2.4.2. Reactome

Reactome (http://www.reactome.org/) is a peer-reviewed database that describes many cellular processes in a single consistent format that links human proteins systematically by function. The pathways documented in Reactome allow for the

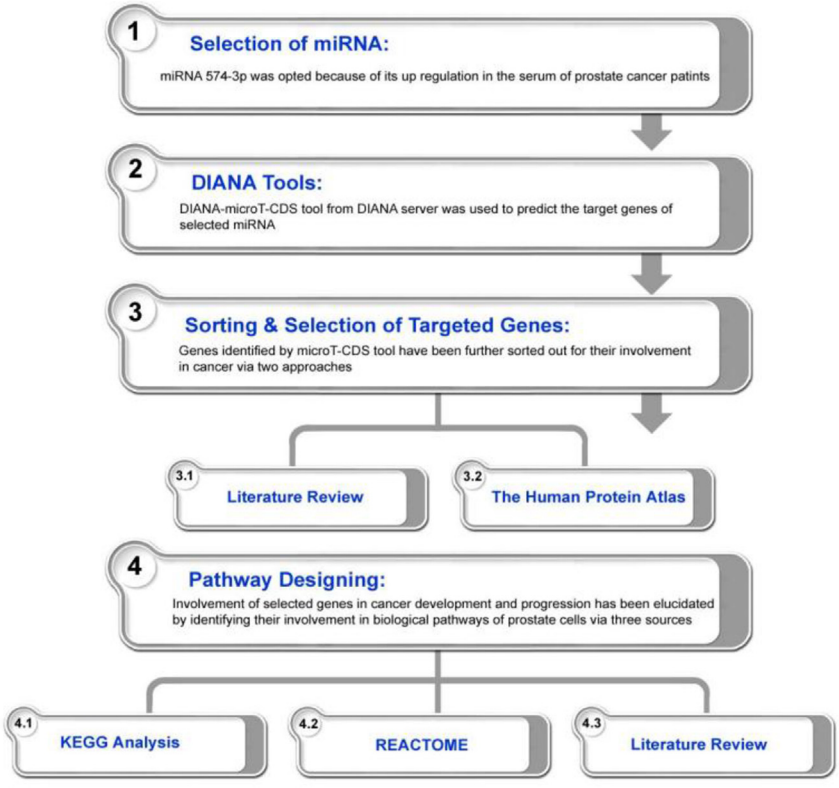

Fig. 1. Illustration of methodology flow sheet.

Table 1
Genes downregulated by miRNA-574-3p in prostate cancer.

Sr.#	Ensemble gene ID	Gene description	Chromosome	Biological process	Annotation source	Expression
1	ENSG00000108094 (CUL2)	cullin 2	10	Cellular response to hypoxia	Reactome; R-HSA-1234176	Prostate, placenta
2	ENSG00000144554 (FANCD2)	Fanconi anemia, complementation group D2	3	Catalyzes the exchange of eukaryotic initiation factor 2-bound GDP for GTP	KEGG, hsa:8890	Prostate, adrenal cortex, amygdala
3	ENSG00000172273 (HINFP)	Histone H4 transcription factor	11	DNA methylation and transcription repression.	KEGG, hsa: 25988.	Brain, heart, skeletal muscle, spleen, prostate
4	ENSG00000198178 (CLEC4C)	C-type lectin domain family 4, member C	12	Antigen-capturing, cell adhesion, cell-cell signaling, glycoprotein turnover, and roles in inflammation and immune response	KEGG, hsa: 170482.	Plasmacytoid dendritic cells (PDCs)
5	ENSG00000196792 (STRN3)	striatin 3	14	Protein phosphatase type 2A complex binding	MIM:614766	Prostate, Colon, B lymphocyte
6	ENSG00000110906 (KCTD10)	potassium channel tetramerization domain containing 10	12	Protein ubiutination	MIM:613421	Prostate gland, tendon, adrenal gland
7	ENSG00000115211 (EIF2B4)	eukaryotic translation initiation factor 2B, subunit 4 delta	2	Regulation of translation	K03680	Prostate gland, testis, buccal mucosa cell
8	ENSG00000010322 (NISCH)	nischarin	3	Cell growth and death, cytoskeleton organization and cell migration	MIM:615507, UniProtKB - Q9Y2I1	Prostate, brain, endocrine tissue
9	ENSG00000237289 (CKTM1B)	creatine kinase, mitochondrial 1B	15	Energy metabolism, Apoptosis of cell	K0093	Prostate, colon, brain

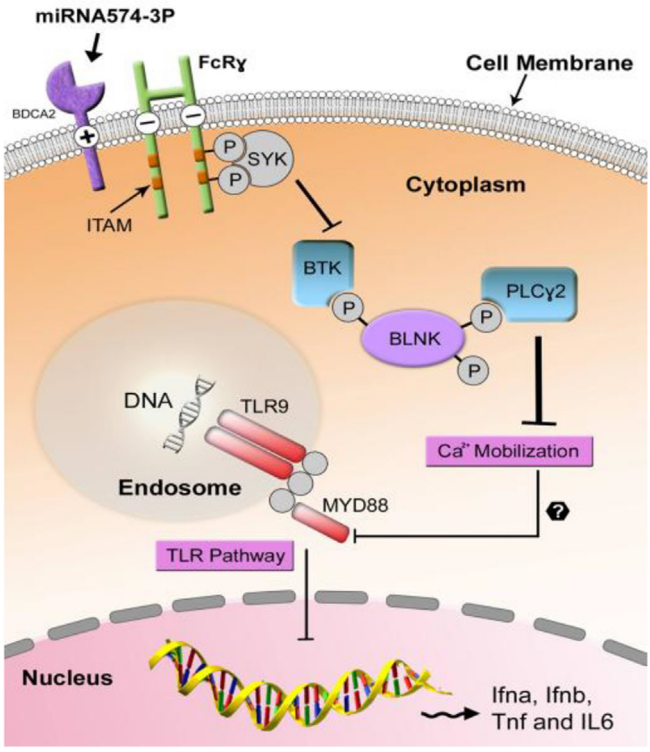

Fig. 2. Illustration of CLEC4C signaling pathway. Downregulation of CLECRC weakens the efficacy of the adaptive immune response to fight modifications, eliminate infected cells, and restrict the tumor-inducing environment.

annotation of a broad range of major disease processes at the molecular level [13].

2.4.3. Literature review

Many shortlisted genes were not found in the pathways retrieved from the KEGG and Reactome databases. The problem was solved by synthesizing the pathways based on available literature.

3. Results

Thirty-nine genes were originally predicted by DIANA-MicroT-CDS to be linked to miRNA-574-3p. We then narrowed the original selection to nine genes that were found to be associated with PCa using literature review and extensive data mining. The targeted genes described in this section are hypothesized to be downregulated in PCa because of high expression of miRNA574-3p and therefore promote the tumor microenvironment and metastasis (Table 1). The involvement of these nine genes in PCa development and progression has been justified through their biological pathways. The results are summarized as follows.

3.1. CLEC4C

Blood dendritic cell antigen 2 protein (BDCA2), also known as CLEC4C, is a member of C-type Lectin receptors (CLRs). CLRs are a large family of soluble transmembrane proteins, which are expressed on monocytes, macrophages, dendritic cells (DCs), etc. and contain one or more C-type lectin-like domains (CTLD). CLEC4C has been recognized as a pattern recognition receptor (PRR) on dendritic cells that interacts with pathogen-associated molecular patterns (PAMPs) and induces cytokine expression [14].

In PCa, higher expression of miRNA-574-3p induces downregulation of CLEC4C, leading to a reduction in the cell's ability to degrade foreign pathogens. Thus, the adaptive immune response is weakened and the body's ability to eliminate infected cells and the tumor-inducing environment is reduced, leading to the downregulation of tumor suppressing genes. This condition creates a persistent tumor-induced tumor suppression environment leading to metastasized PCa (Fig. 2).

3.2. HINFP

HINFP, also known as MIZF (MBD2 interacting zinc finger protein), encodes a transcription factor that interacts with methyl-CpG-binding protein-2 (MBD2), a component of the MeCP1

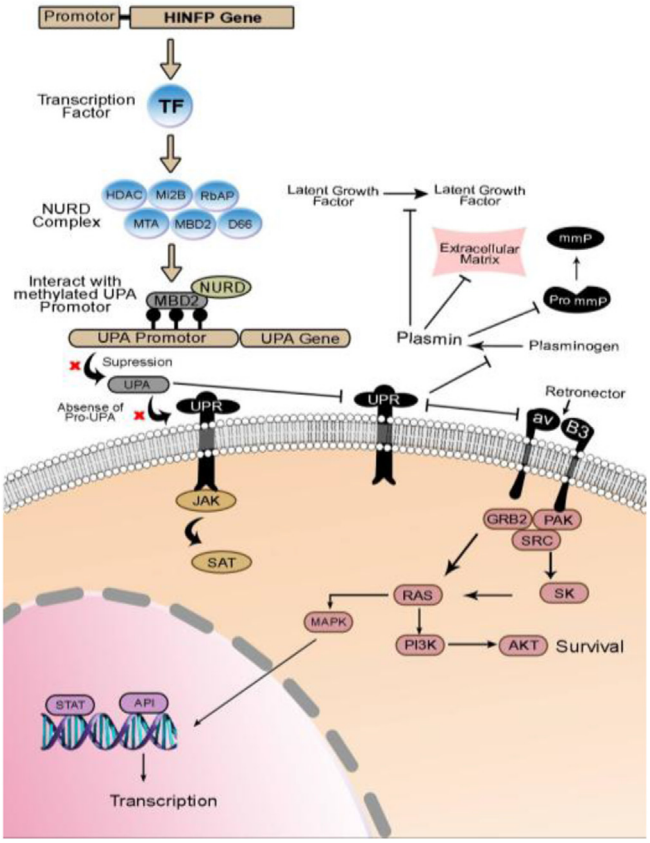

Fig. 3. Illustration of HINFP signaling pathway. High expression of uPA induces invasion, angiogenesis, and metastasis in several tumor malignancies including prostate cancer.

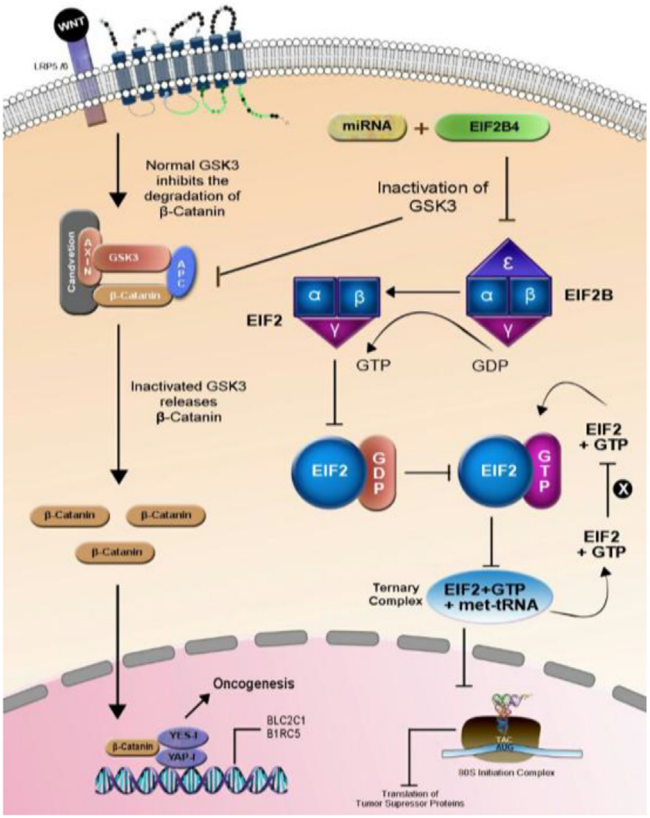

Fig. 4. Illustration of EIF2B4 pathway. Downregulation of EIF2B4 caused the inactivation of GSK-3 and enrichment of Wnt signaling pathway, which is beneficial for tumor growth.

histone deacetylase (HDAC) complex [15]. This interaction induces DNA methylation, inhibiting gene expression by transcriptionally silencing and preventing the binding of transcription factors to the regulatory sequences of the promoter. DNA methylation also allows for the binding of methylcytosine protein binding complexes, which recruit HDAC and cause chromatin condensation and repression of transcription.

Urokinase plasminogen activator (uPA) induces growth, invasion, angiogenesis, and metastasis in several tumor malignancies including PCa [16]. Methylation of CpG islands by the HDAC complex within the uPA promoter is a crucial molecular mechanism that inactivates the transcription of the uPA gene and prevents the progression of PCa (Fig. 3). A close review of the available literature shows that uPA is highly expressed in invasive PC3 cells when the uPA promoter is unmethylated [17].

3.3. EIF2B4

EIF2BF, eukaryotic initiation factor 2B, plays an important role in translation. It is encoded by transcription factor EIF2B4 and consists of up to five subunits EIF2Bα, EIF2Bβ, EIF2Bε, EIF2Bγ, and EIF2Bδ and acts as a GTP exchange factor (GEF) during regulation of mRNA translation [18,19].

In normal individuals, transcription factor EIF2B4 is related to the cellular stress response. In PCa, overexpression of miRNA-574-3p causes downregulation of EIF2B4 and induces suppression of EIF2B, which inhibits protein synthesis and allows for Tumor-associated macrophage (TAM) activation. TAMs can independently synthesize cytokines necessary for survival, growth, and proliferation [20] (Fig. 4).

3.4. KCTD10

Potassium channel tetramerization domain containing 10 (KCTD10) is a member of the polymerase delta-interacting protein 1 (PDIP1) family and contains a conserved BTB domain [21]. KCTD10 is involved in development during the embryonic stages by interacting simultaneously with Cullin3 and Notch signaling proteins [22]. Notch signaling is responsible for the regulation of some cellular cascades (e.g., cell survival, proliferation, and angiogenesis) [23], and Cullin3 is involved in ubiquitin-dependent proteasomal degradation [24]. KCTD10 negatively regulates Notch signaling by proteolytic degradation of Notch1 receptors [25].

Quantitative RT-PCR of RNA extracted from PCa cell lines shows that Notch1 receptors are upregulated in prostatic epithelial cells [26]. Immunohistochemical analysis of the Notch ligand Jagged1 showed a higher level of Jagged1 in metastatic prostate tissue [27]. Notch1/Jagged1 signaling promotes progression of PCa [28]. Hence, it may be concluded that downregulation of KCTD10 by miRNA574-3p ultimately leads to aggressive invasion by PCa cells (Fig. 5).

3.5. CUL2

CUL2 belongs to the cullin protein family, which is responsible for regulating proteasomal degradation [29]. Furthermore, CUL2 specifically mediates ubiquitin-mediated degradation of HIF1α [30]. Under normoxic conditions, HIF1α is degraded following hydroxylation of specific proline residues. This degradation creates a binding site for VHL and allows for ubiquitination and the

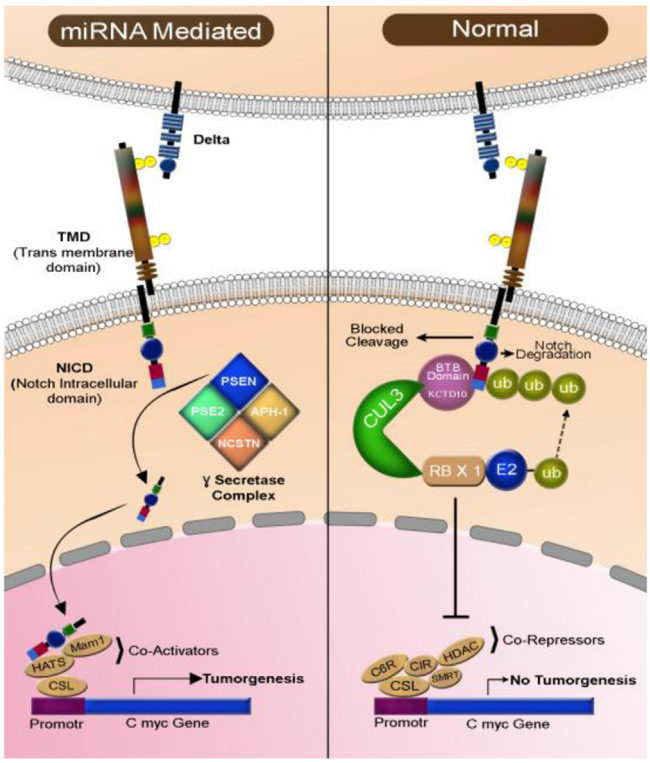

Fig. 5. Illustration of KCTD10 signaling pathway. In the nucleus, the Notch domain binds with co-activator of oncogenes (c-myc) and regulates transcription, thus promoting tumorigenesis.

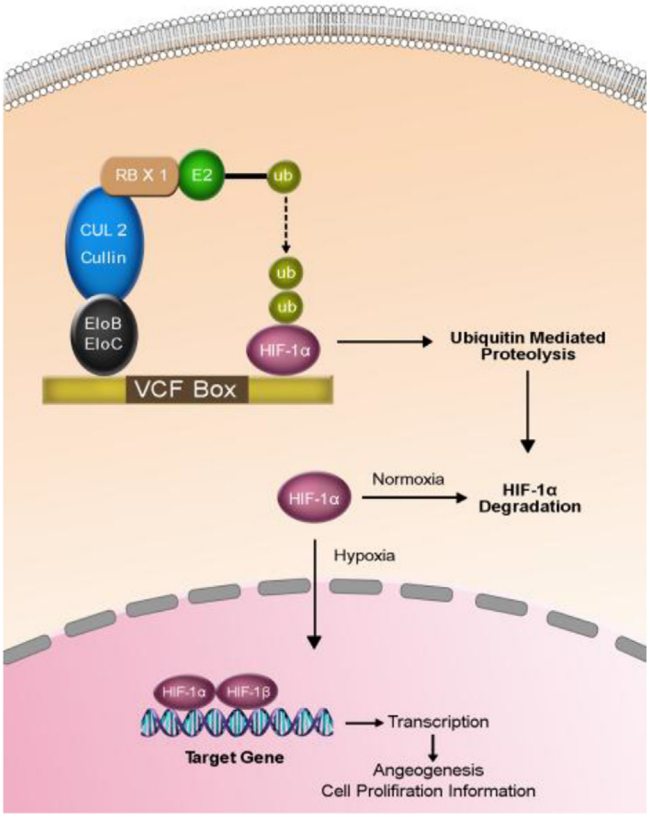

Fig. 6. Illustration of the CUL2 signaling pathway. In cancerous cells under hypoxia conditions, HIF-1α becomes stable and translocates to the nucleus where it binds to HIF-1β and interacts with a co-activator of targeted oncogenes, thus promoting angiogenesis and inflammation.

consequent prevention of abnormal angiogenesis. Conversely, the random growth of tumor cells may lead to hypoxia due to low blood circulation [31]. Under hypoxic conditions, HIF1α translocates to the nucleus, binds HIF1β, and forms an HIF1 transcriptional complex [30]. This complex ultimately triggers expression of genes involved in angiogenesis, which provides a favorable environment for the growth of cancer cells. It can be concluded from our study that the suppression of CUL2 by miRNA574-3p will block proteolytic cleavage of HIF1α and favor the survival and progression of cancer cells (Fig. 6).

3.6. STRN3

STRN3 belongs to the striatin family and forms the regulatory subunit of the protein phosphatase 2A complex (PP2A). PP2A is a conserved Ser/Thr phosphatase involved in regulation of all signaling processes [32,33]. It is a heterotrimeric complex consisting of a catalytic subunit (PP2AC), adaptor subunit (PP2AA), and a regulatory subunit (PP2AB) [34]. Interestingly, PP2A has been shown to play a regulatory role in the MAP kinase pathway, Wnt signaling pathway, cell cycle arrest, and many other signaling cascades [35].

PP2A dephosphorylates Raf, positively regulating the MAPK pathway [36]. Raf mediates phosphorylation of c-myc at the Thr58 position through downstream MEK/ERK activation, making c-myc susceptible to ubiquitin-mediated degradation [37]. In cancerous cells, miRNA54-3p reduced the expression of STRN3, causing the heterotrimeric complex PP2A to dissociate. Thus, phosphorylation of c-myc at Ser62 causes decreased differentiation and increased proliferation [38].

PP2A also negatively regulates Wnt signaling by activating GSK3β. GSK3β is essential for the degradation of β-catenin. The complex that degrades β-catenin includes APC, GSK3β, and the axin proteins. This complex phosphorylates free cytoplasmic β-catenin, marking it for ubiquitin-mediated degradation [39]. Activation of the Wnt pathway leads to reduced phosphorylation of β-catenin by the APC-axin-GSK3β complex. β-catenin accumulates and translocates itself to the nucleus where it associates with the T-cell factor (TCF) and lymphoid enhancer factor (LEF). β-catenin activates transcription of the target genes, which includes c-myc, c-jun and fra-1 [40]. Thus, overexpression of miRNA574-3p may lead to the downregulation of PP2A, resulting in enhanced tumorigenesis. Furthermore, PP2A has also been reported to regulate stress response proteins such as ataxia-telangiectasia mutated (ATM) kinase and ataxia-telangiectasia related (ATR) kinase [41]. These kinases further phosphorylate the cell cycle checkpoint proteins, resulting in activation of p53 and arresting the cell at the G_0 phase [42]. Therefore, it can be concluded that low expression of PP2A may induce the tumor microenvironment by triggering the cell cycle (Fig. 7).

3.7. CKTM1B

CKMT1B is a protein-coding gene that encodes ubiquitous mitochondrial creatine kinase (U-MtCK). This kinase has a profound role in the transfer of high-energy phosphates to the creatinine in the mitochondria. Creatinine is a cytosolic carrier and belongs to the creatine kinase isozyme family. Its cellular location is in the mitochondrial intermembrane space, where it phosphorylates creatine and converts it to phosphocreatine through an energetically favorable process. Phosphocreatine is then able to

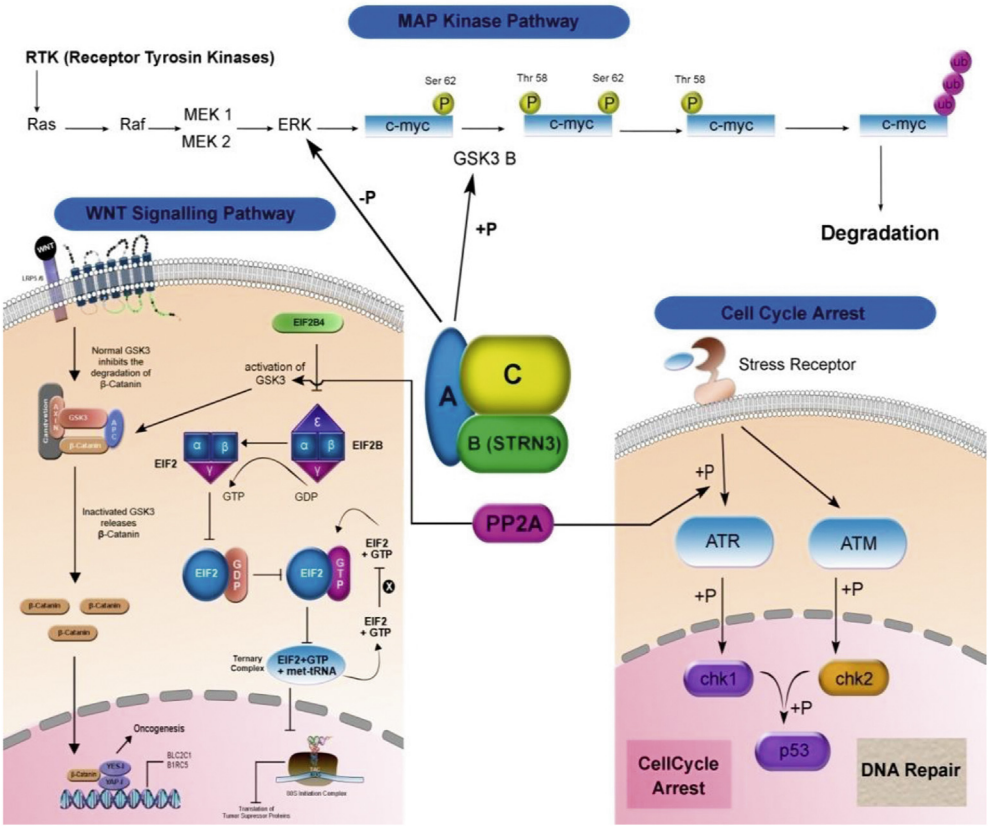

Fig. 7. Illustration of the STR3 signaling pathway. In cancerous cells, all these cycles terminate because the PP2A complex can't assemble properly due to blockade of STRN3 by miR574-3p. In the absence of the PP2A complex, signaling cascades lead to tumorigenesis.

diffuse into the cytosol where it is used in various high-energy metabolic pathways [43].

In tumor cells, where the CKMT1B expression has been downregulated by miRNA574-3p, degradation of poly (ADP-ribose) polymerase (PARP) protein is observed [44]. PARP plays a key role in DNA damage repair and caspase-3 activation pathways, and therefore its deregulation may lead to cancer development in prostate cells (Fig. 8).

3.8. FANCD2

The FANCD2 gene encodes a Fanconi anemia group D2 protein that plays an important role in DNA damage repair and contributes to homologous recombination [45]. It is a major part of the Fanconi anemia (FA) pathway [46]. Activation of the FA pathway by ATM (Ataxia telangiectasia mutated) and ATR (Ataxia telangiectasia Rad3) kinases plays an important role in DNA damage repair [47]. These kinases are activated by single strand or double strand DNA damage, as well as phosphorylated cancer suppressing genes like p53 [48]. The ubiquitinated FANCD2 and FANCI complexes, along with other FA proteins, are translocated towards DNA damage sites and recognize the damage. After the repair process, USP1 associated factor (UAF1) and ubiquitin-specific protease (USP1) deubiquitylate FANCD2 and FANC1, and the complex is released from DNA [49]. When the FANCD2 gene is downregulated, it may halt DNA repair in cells, leading to cancer (Fig. 9).

3.9. NISCH

The NISCH gene encodes noradrenergic imidazoline-1 receptor (I1R) protein, which is localized to the plasma membrane [50]. This protein binds favorably to the cytoplasmic domain of the integrin α5 subunit (Iα5β1) and prevents cell migration by altering actin filament organization in cancerous cells. Nischarin interacts with LKB1 tumor suppressor protein at the N and C-termini of the kinase domains PAK1, PDZ, and LIMK1 to form a scaffold protein. This scaffold regulates the function of LKB1 in two ways. First, it translocates LKB1 from the nucleus to the cytoplasm where it regulates cell polarity events and activates the kinases responsible for regulating LKB1 activity. LKB1 co-localizes with Cdc42 and PAK, both of which play significant roles in downstream cellular polarity events. Hyperphosphorylation of these proteins occurs due to the downregulation of tumor suppressor proteins such as Nischarin and LKB1, resulting in increased frequency of cell polarity events [51]. Downregulation of Nischarin protein by miRNA prevents interactions between NISCH and LKB1 and phosphorylates downstream targets PAK1 and LIMK1, both of which regulate the G_2/M checkpoints in the cell cycle [52]. Interruption of this cascade raises the cellular polarity events and enhances tumor cell migration, tumor growth, and the invasive characteristics of metastatic cancer development (Fig. 10).

4. Discussion

Many studies have shown that aberrant expression of miRNA574-3p is associated with PCa and is found to be upregulated in the plasma, urine, and serum exosomes at stage 3 and 4 in PCa patients [7,53]. However, the mechanisms linking miRNA574-3p to PCa are still unclear [54]. In this study, we outline the network of genes targeted by miRNA 574-3p and its possible genotoxic effects

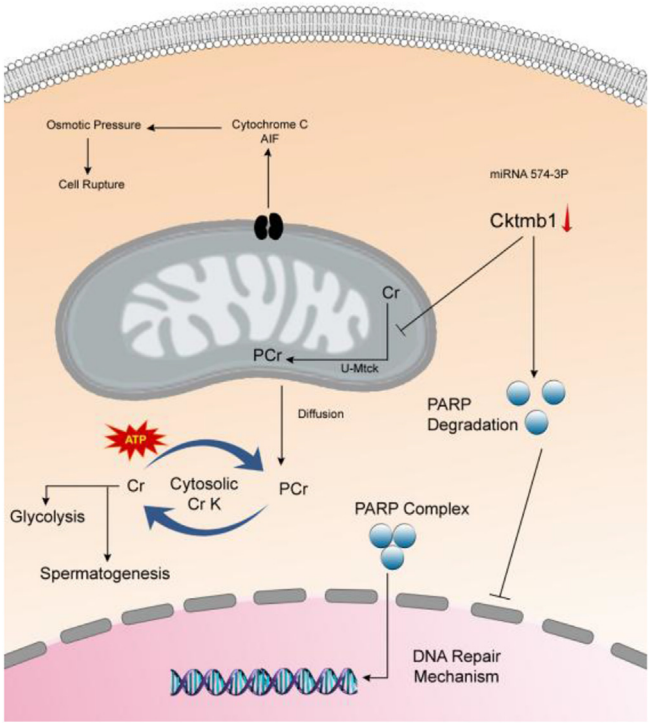

Fig. 8. Illustration of CKTM1B signaling pathway. Depolarization leads to cells rupturing due to increased osmotic pressure. In this way, healthy cell necrosis twitches that ultimately initiates the cancerous cell's development.

on prostate cells. These genotoxic effects may promote the formation of the tumor microenvironment, affecting normal homeostasis through several pathways.

CELC4C is found to be involved in the regulation of the immune system [14]. Several studies provide evidence that compromising the health of the immune system may lead to the development and progression of PCa. Negative regulation of CLEC4C leads to suppression of several adaptive immune responses against pathogens, mutations, elimination of infected cells, and the tumor-inducing environment [55].

The KCTD10, STRN3, and EIF2B4 genes are downregulated due to the upregulation of miRNA 574-3p in PCa, which allows for the activation of several signaling cascades such as the Notch signaling pathway and the Wnt signaling pathway. Several experimental studies show that KCTD10 negatively regulates the Notch signaling pathway, which is responsible for the regulation of other cellular cascades (e.g., cell survival, proliferation, angiogenesis, and metastasis) [56]. Therefore, downregulation of KCTD10 may influence metastasized PCa. We demonstrate that the downregulation of EIF2B4 and STRN3 may cause the inactivation of GSK-3 and the enrichment of the Wnt signaling pathway by stabilizing β-catenin. Enrichment of Wnt also confirms the role of miRNA574-3p in the proliferation of prostate cells.

FANCD2 and CKMT1B are suppressed by miRNA574-3p, which halts the DNA repair pathway in prostate cells. Altered expression of these genes disrupts the function of cell cycle checkpoint proteins and favors the progression of cancer. These findings also support the conclusion that the DNA damage response is critical for the survival of cancerous cells [57].

Interestingly, our data suggest that CUL2, HINFP, and NISCH promote tumor survival and angiogenesis in PCa. Downregulation of CUL2 causes HIF-α to escape degradation, leading to angiogenesis in prostate cells and a reduction of hypoxia in tumors [30].

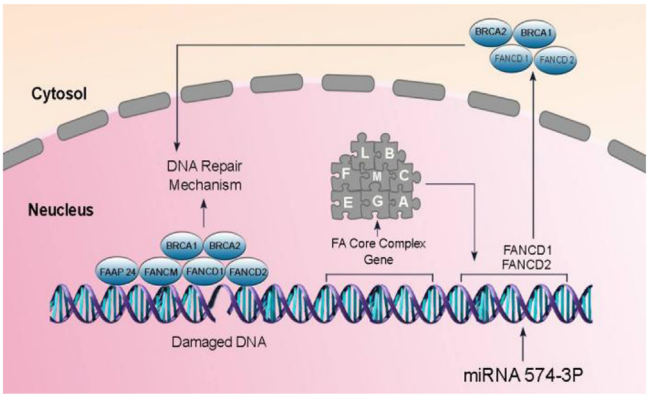

Fig. 9. Illustration of the FANCD2 signaling pathway. Downregulation of FANCD2 negatively affects the DNA repair mechanism, a hallmark of cancer development.

Pathway analysis also leads us to the assumption that downregulation of NISCH influences PCa progression by promoting tumor cell migration and invasion by hyperphosphorylation of tumor suppressor genes. Also, we identified that the miRNA574-3p target gene, HINFP, is involved in the methylation of urokinase plasminogen activator (uPA), which is a known oncogene. Downregulation of HINFP results in activation of uPA, causing PCa invasion, angiogenesis, and metastasis.

Pathway analysis of these nine target genes uses a standardized approach to achieve meaningful and verifiable results regarding the oncogenic function of miRNA 574-3p in prostate cells. This study provides a clear picture of the underlying cellular mechanisms and molecular pathways that contribute to PCa progression and metastasis. The results presented above will aid in the identification and design of new therapies for PCa with better treatment efficacy.

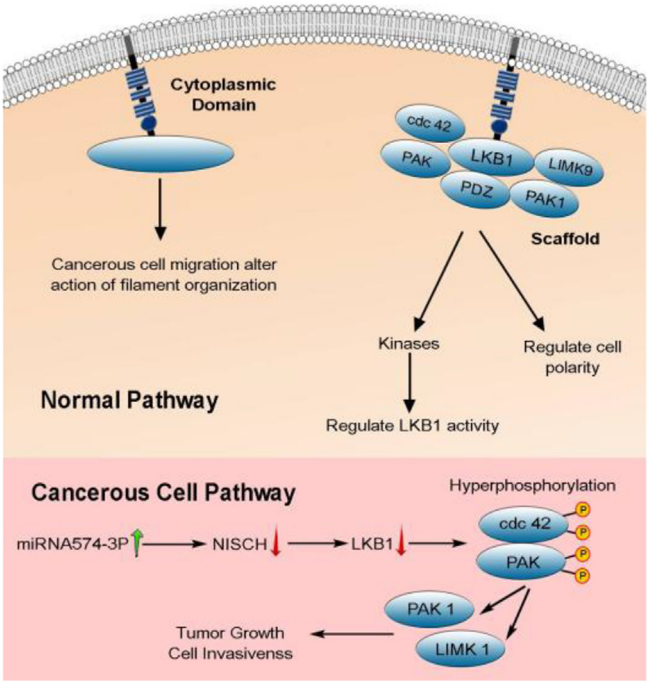

Fig. 10. Illustration of NISCH signaling pathway. Downregulation of Nischarin by miRNA-574-3p, results in hyperphosphorylation of downstream targets PAK1 and LIMK1.

5. Conclusion

From our study, we conclude that the upregulation of miRNA 574-3p in PCa cells favors the tumor microenvironment and metastasis. miRNA 574-3p targets several genes that have been associated with normal body regulatory processes. Furthermore, pathway analysis also reveals the involvement of the target gene in the regulation of several signaling cascades such as Notch signaling pathway, Wnt signaling pathway, MAP kinase pathway, DNA damage response, apoptosis, G1 to S cell-cycle control, angiogenesis, inflammatory response pathway, translation factors, and the expression of oncogenes. These findings illustrate the potential of miRNA574-3p as a candidate for novel PCa therapeutics.

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