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PYGO2 regulates IL10 and plays immunosuppressive role through ESCC progression

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Abstract

Background Esophageal squamous cell carcinoma (ESCC), one of the most aggressive carcinomas of the gastrointestinal tract, is the sixth most common cause of cancer-related death. Wnt pathway plays a pivotal role in cell proliferation and differentiation. *PYGO2* and *IL10* are involved in this pathway. Our aim in this study was to examine the correlation between *PYGO2* and *IL10* expression in ESCC patients and cell lines.

Methods Relative-comparative real time-PCR (RT-qPCR) was used to evaluate the *PYGO2* and *IL10* mRNA expression profile in 58 non-treated ESCC compared to their margin normal tissues. Expression of *PYGO2* was induced in KYSE-30 and YM1 ESCC lines and *IL10* expression was analyzed.

Results The results revealed the significant overexpression of *PYGO2* and *IL10* mRNA in 31.0% and 51.7% of ESCCs, respectively. The *PYGO2* and *IL10* overexpression was significantly correlated to each other ($p=0.007$). Concomitant overexpression of the genes was significantly associated to grade of tumor differentiation ($p<0.01$), and tumor depth of invasion ($p<0.05$). Induced expression of *PYGO2* caused a meaningful change in *IL10* expression in ESCC cells.

Conclusion *PYGO2* may regulate *IL10* through Wnt/β-catenin signaling pathway, suggesting a possible oncogenic role for *PYGO2/IL10* axis in ESCC tumorigenesis. Considering the involvement of *IL10* as an anti-inflammatory cytokine and *PYGO2* role in elevated tumor invasion and metastasis, possible functional interaction between these factors may promote a process which induces invasion and malignant phenotype in ESCC.

Keywords Co-overexpression, *PYGO2*, *IL10*, Quantitative real-time PCR, Wnt/β-catenin pathway

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Introduction

Esophageal cancer (EC) is the 8th most prevalent malignancy and 6th leading cause of cancer-related death worldwide. It can be histopathologically categorized to two common subtypes including esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). ESCC as the predominant subtype of EC, accounts for nearly 90% of EC particularly in Iran, North China, and Africa. Due to late diagnosis, remote metastasis, and local invasion, ESCC has a quite poor 5-year survival rate (15–25%), and patients have to relinquish the principal curative option of surgical resection [1, 2].

Deregulation of signaling pathways, such as canonical Wnt/β-catenin, is associated with the progression and development of various malignancies. Binding of specific Wnt ligands to membrane receptors/co-receptors induces inactivation of an *AXIN-APC-GSK3β* destruction complex which results in stabilization and accumulation of *β-catenin* in the cytoplasm. Consequently, *β-catenin* is translocated into the nucleus and binds to *TCF*/*LEF* transcription factors to activate transcription of Wnt target genes [3]. Pygopus2 (*PYGO2*), which contains a highly conserved C-terminal plant homeo domain (PHD), functions as a significant coactivator of the Wnt/β-catenin transcriptional complex (*LEF*/*TCF*). *PYGO2* directly bind to histone H3 trimethylated at lysine 4 (H3K4me3), an epigenetic mark linked to transcriptional activation, through its PHD and activates β-catenin-dependent transcriptional regulation [4]. Due to *PYGO2* substantial regulatory roles in the cell, its deregulation may lead to tumorigenesis. Pivotal role of *PYGO2* has revealed in multiple cancers including ESCC, ovarian, breast, cervical, colon, lung, and liver malignancies.

According to contribution of chronic inflammation in EC susceptibility, several involved genes in inflammatory pathways may play role in EC development. Interleukin (*IL*)*-10*, an immunosuppressive T helper 2 type cytokine, is secreted by various types of cells including B cells, dendritic cells, monocytes/macrophages, diverse T regulatory cell subsets, $CD8^+$ and $CD4^+$ T cells and natural killer (NK) cells. *IL10* plays diverse roles in the regulation of immune activities such as inhibition of T helper 1 type cytokine production and T lymphocyte proliferation, blunting of cytotoxic responses, and impairment of antigen presentation cells. The regulation of *IL10* expression appears to be intricate, so that it can function as either a pro-tumorigenic or an antitumor agent [5]. However, upregulation of *IL10* expression has verified in a number of hematopoietic and solid tumors including ESCC, colon, breast, and lung cancers [6, 7]. The Wnt/β-catenin signaling pathway applies both proinflammatory and anti-inflammatory functions. There was a remarkable correlation between enhancing the level of Wnt pathway ligand/receptor (*Wnt3a*/*FZD9*, respectively) and increased level of *IL10* in enteric nervous system [8]. *IL10* has likewise been demonstrated as a *β-catenin* target gene in colon cancer [9]. These findings support the involvement of IL10 in Wnt/β-catenin pathway active state.

Considering the aberrant activation of Wnt/β-catenin signaling pathway in progression and metastasis of multiple cancers, as well as involvement of *PYGO2* and *IL10* gene expression in ESCC development, we aimed in the present study to evaluate the probable correlation between *PYGO2* and *IL10* expression in ESCC patients and cell lines.

Materials and methods

In silico study

The GeneMANIA web-based database was employed to predict potential targets of PYGO2-IL10 through key genes of the Wnt/β-catenin signaling pathways (https://genemania.org/). Additionally, the Phenolyzer website was utilized to predict interactions between PYGO2-IL10, as well as key genes in the Wnt/β-catenin pathway, within the context of ESCC (https://phenolyzer.wglab.org/).

Cell lines and culture condition

KYSE-30 and YM1 ESCC lines were obtained from Pasteur Institute of Iran (Tehran, Iran), and cultured in RPMI-1640 and DMEM culture mediums (Bio-Idea, Iran) supplemented with 10% fetal bovine serum (FBS solution, BI, USA), and 100 µg/ml penicillin/streptomycin (Gipco, USA), and incubated in %90 humidity incubator containing %5 CO2 at 37 °C.

Transfection

The Human *PYGO2* ORF expression plasmid, C-HA tag (Sino Biological Inc. Catalog Number: HG20275-CY) was applied to enforce *PYGO2* expression in the cells. Transfection was performed using Lipofectamin 2000 transfection reagent (Roche, Basel, Switzerland). Nearly 600,000 cells were seeded per six-well plate and transfected with a total of 2 µg plasmid. The transfection efficiency was checked [10]. After 48 h cells were treated with trypsin and EDTA and subjected to RNA extraction.

ESCC patients and tissue samples

The fresh tumoral and para-tumor normal tissues were collected from 58 ESCC patients who underwent surgical removal of tumor tissue in Omid Hospital of Mashhad University of Medical Sciences (MUMS). These patients had not received radiotherapy or chemotherapy before the surgery. All patients provided the informed consent and the study was approved by the Ethics Committee of MUMS (No.88098). According to the latest Union International Cancer TNM classification guidelines, the histopathological features of the samples including tumor size,

nodal involvement, and metastasis (TNM) staging were determined, then samples were confirmed to be tumor or normal histologically.

RNA extraction, cDNA synthesis and quantitative RT-PCR

RNA was isolated from all tissues, using TriPure RNA extraction reagent (Roche, Nutley, NJ). The RNA integrity in all samples were verified by agarose gel electrophoresis. The cDNA was synthesized from RNA with oligo $(dT)_{18}$ and random hexamer primers concurrently. Relative comparative real-time PCR for *PYGO2* and *IL10* genes were performed with peculiar primer sets (Table 1) using SYBR Green method, containing ROX as a reference dye in a Stratagene Mx3000P real-time thermocycler detection system (Stratagene, La Jolla, CA, USA). The thermal cycling program included an initial denaturation step of 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at 62 °C, and 45 s at 72 °C. All samples were tested in triplicate. For the normalization of RNA input, Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was employed as an endogenous control, which displays the lowest mutability in expression in esophageal tissues [11]. The ΔΔCt method was employed to evaluate fold change of gene expression, as described before.

Statistical analysis

The statistical analyses were performed using SPSS 19.0 statistical package (SPSS, Chicago, IL, USA). The χ^2 or Fisher's exact test, independent-sample t test and ANOVA were utilized to analyze associations between gene expression and diverse histopathological factors. The correlation between *PYGO2* and *IL10* expression was analyzed using Pearson's correlation. A p-value of < 0.05 was defined as statistically significant.

Results

PYGO2-IL10 pro-pro interaction with key genes of Wnt signaling pathway

In this study, we performed comprehensive functional analyses to investigate the interaction between PYGO2-IL10 and key genes involved in the Wnt signaling pathway. To assess the interactive relationships, we utilized open-source expression analysis tools, including GeneMania25 and phenolyzer. Figure 1a illustrates the results of the interactive analysis, focusing on PYGO2-IL10, and the expression of Wnt signaling pathway genes. GeneMANIA analysis revealed significant protein-protein interactions between PYGO2 and a variety of Wnt signaling proteins, including CREBBP, BCL9, CTNNB1, KMT2D and PYGO1. Notably, pivotal protein-protein interactions were also observed between IL10 and SOX2, PTK7, IL26, IL19, IL20 and IL10RA, as depicted in Fig. 1.

Furthermore, phenolyzer analyses were conducted to explore the contextual associations of PYGO2-IL10 with various Wnt signaling pathway key genes in the context of ESCC. The results obtained from phenolyzer analyses (Fig. 1d) highlighted a multitude of significant cellular associations linking PYGO2-IL10 with these genes. These findings provide valuable insights into the functional interactions of PYGO2-IL10 with key genes within the Wnt signaling pathway.

Table 1 Primer sequences used for comparative real-time RT-PCR

Gene	Forward	Reverse	Product size (bp)
IL10	AACCAAGACCCAGACATCAAGG	CATTCTTCACCTGCTCCACG	136
PYGO2	GTCCCCCACTCCATGGCCGCCTCG	TCGCTTCTTTTCTGGACTCTTC	147
GAPDH	GGAAGGTGAAGGTCGGAGTCA	GTCATTGATGGCAACAATATCCA	101

ESCC patients and tissue samples

Primary frozen tumor tissues and their margin non-tumorous esophagus were obtained from 58 newly diagnosed ESCC patients prior to receive radio- or chemo-therapy. Thus, the histopathological characteristics of ESCC tissues were not affected by subsequent therapeutic interventions. The male-to-female ratio of enrolled patients in this study was 1.2 (32:26). The mean age ± standard deviation (SD) of patients was 61.85 ± 12.25 (age range 30–83 years). The size of tumor samples ranged from 0.5 to 12 cm (mean ± SD: 4.01 ± 1.93), resected from middle or lower parts of the esophagus. The clinicopathological features of the patients are listed in Table 2.

Upregulation of *PYGO2* and *IL10* in ESCC

Relative comparative real-time PCR (qRT-PCR) was used to analyze *PYGO2* and *IL10* expression in 58 ESCC patients. Both genes were significantly overexpressed at mRNA level in 18 of 58 (31.0%) and 30 of 58 (51.7%) ESCC tumors compared to paired adjacent non-tumor tissues, respectively. The mean (± SD) of *PYGO2* mRNA expression fold changes in ESCC tissues was 1.02 (± 2.18), with minimum and maximum fold changes of -3.31 and 7.00, respectively. The mean (± SD) of *IL10* fold changes in ESCC tissues was 1.81 (± 2.39). Its fold changes was ranged from − 4.50 to 6.03 fold, in ESCC samples. Figure 2 visualizes the profile of *PYGO2* and *IL10* gene expression in ESCC patients as a scatter plot.

Association between *PYGO2* and *IL10* gene expression

The *PYGO2* and *IL10* expression were significantly correlated to each other in ESCC samples ($p = 0.007$,

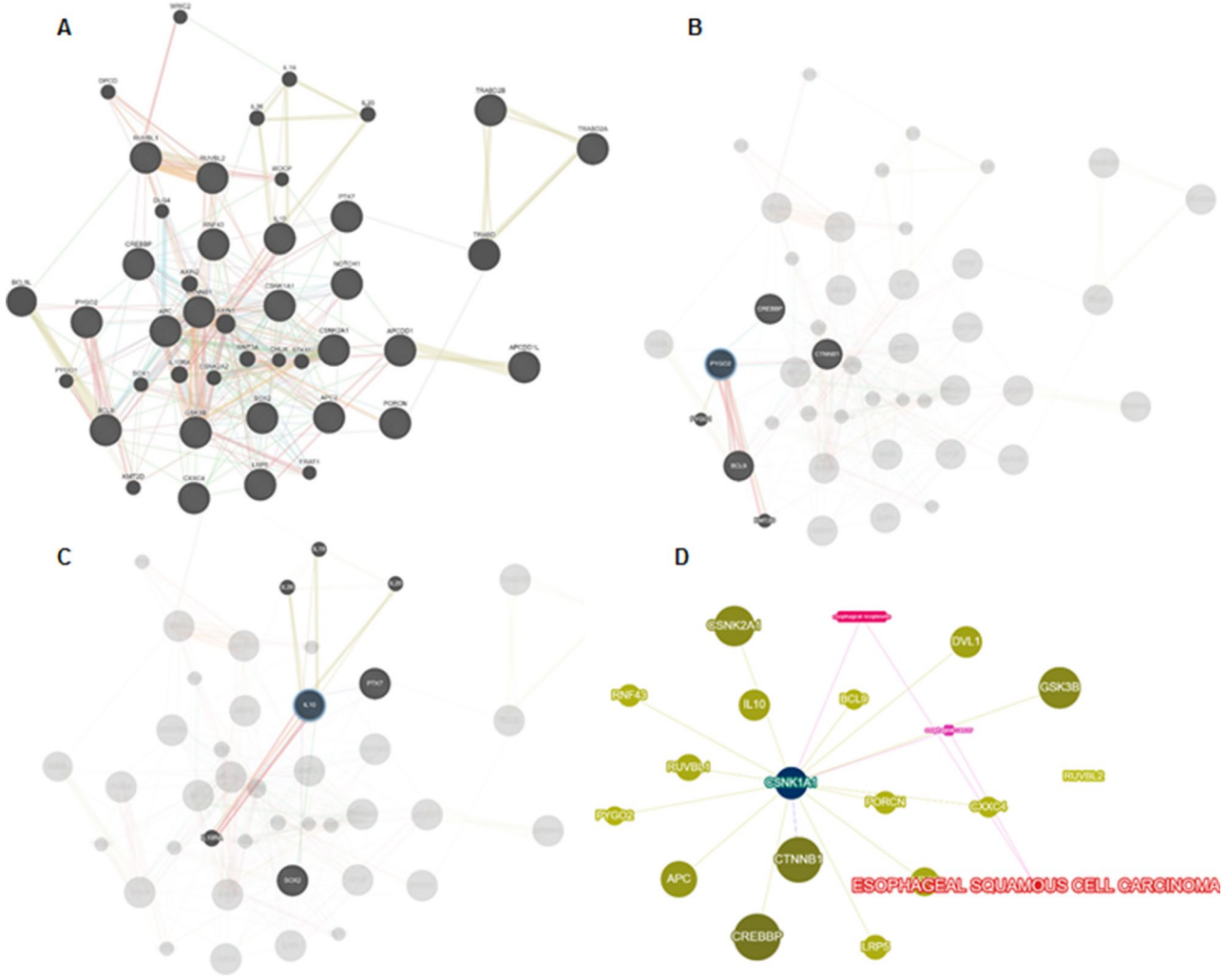

Fig. 1 (**a**) The predicted protein-protein interaction network generated using GeneMANIA, illustrating the predicted relationships between PYGO2-IL10 through Wnt signaling pathway genes. (**b**) The protein-protein interaction network for PYGO2. (**c**) The protein-protein interaction network for IL10. (**d**) The predicted protein-protein interaction network in the context of ESCC, based on predictions from Phenolyzer

correlation coefficient = 0.385). Regression plot depicting this correlation is presented in Fig. 3. Intriguingly, gene expression data from ESCC samples indicated the overexpression of both genes in 24.13% (14 of 58) of patients, whereas 34.48% (20 of 58) of specimens had high mRNA level of at least one gene. Neither *PYGO2* nor *IL10* was overexpressed in 41.37% (24 of 58) of tumors (Table 3).

Association of the genes with surgical stage

The overexpression of *PYGO2* and *IL10* was associated to each other in higher-stage of the disease (stage III; $p < 0.01$). Indeed, 7 of 18 tumors (38.88%) that overexpressed *PYGO2* and 9 of 30 tumors (30.0%) that overexpressed *IL10* showed stage III of tumor progression. While 36% (21 of 58) of tumors with advanced stage of disease displayed significant co-overexpression of *PYGO2* and *IL10* ($p < 0.001$), other cases with lower tumor stages (I and II) did not manifest co-overexpression of the genes ($p = 0.189$).

Association of the genes with lymph node metastasis

Interestingly, we found significant correlation between expression of the genes in metastasized tumor cell into the lymph nodes ($p = 0.001$). Moreover, *PYGO2* overexpression was significantly associated with tumor metastasis to lymph nodes ($p = 0.017$). Among 25 patients with node metastasis, 7 patients (28%) had *PYGO2* overexpression. However, in 66.66% of patients (22 of 33) without any metastasis to lymph nodes, *PYGO2* was not overexpressed. There was no significant association between *IL10* overexpression and lymph node metastasis.

Association of the genes with depth of tumor invasion

The expression pattern of both genes was significantly associated to each other in ESCC patients with invaded

Table 2 Correlations between *PYGO2* and *IL10* gene expression and clinicopathological characteristics of ESCC patients

		PYGO2		IL10	
Clinicopathology		***N/U****	**O**	***N/U***	**O**
Sex	Male	21	11	15	17
	Female	19	7	13	13
Node metastasis	No metastasis	22	11	13	20
	Node metastasis	18	7	15	10
Depth of invasion	T1 and T2	8	3	6	5
	T3 and T4	32	15	22	25
Stage of progression	Stages I and II	26	11	16	21
	Stages III and IV	14	7	12	9
Grade of differentiation	P.D**	6	5	5	6
	M.D	25	10	16	19
	W.D	9	3	7	5
Tumor location	Lower	14	9	10	13
	Middle	25	9	17	17
	Upper	1	0	1	0

*N/U: Normal/Underexpression, O: Overexpression

**P.D: Poorly differentiated, M.D: Moderately differentiated, W.D: Well differentiated

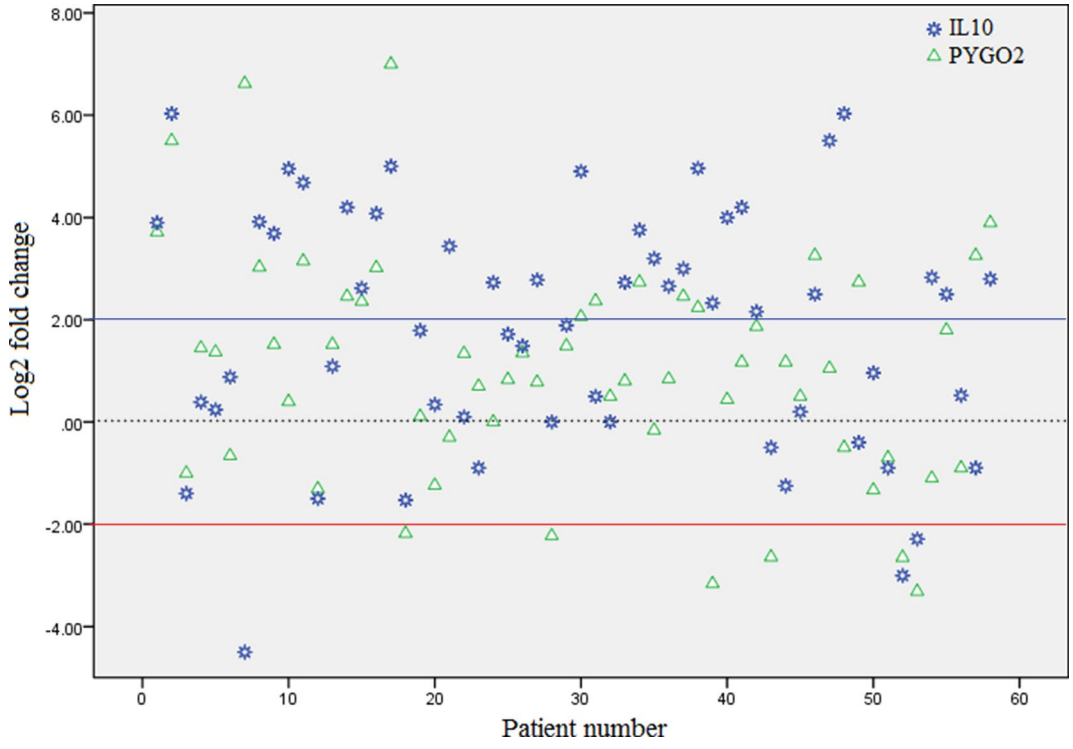

Fig. 2 Descriptive analysis of *PYGO2* and *IL10* gene expression pattern in ESCC patients as scatter plot. The *Y*-axis represents the log 2 fold change of gene expression, and the *X*-axis indicates the number of patients. Relative mRNA expression of more than two-fold in tumor tissue is considered as overexpression; less than minus two-fold as underexpression and the range in between is defined as normal or no change

tumors to the adventitia (T3; $p = 0.031$). Concomitant overexpression of the genes was significantly correlated with depth of tumor invasion ($p = 0.032$). Overexpression of *IL10* was also significantly correlated with depth of tumor invasion ($p = 0.020$). Indeed, 25 of 47 (53.19%) tumors with invasion to adventitia (T3), showed *IL10* overexpression.

Association of the genes with grade of tumor differentiation and location

We found a significant association between *PYGO2* and *IL10* expression in moderately differentiated tumor samples ($p = 0.001$), but not in patients with well and poorly differentiated tumors ($p = 0.102$). Co-overexpression of *PYGO2* and *IL10* was displayed in 9 of 35 (25.7%)

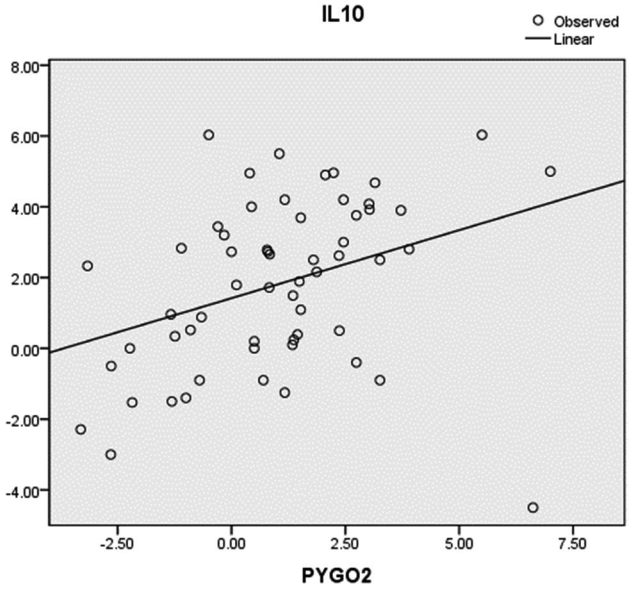

Fig. 3 Regression plot representing correlation between *PYGO2* and *IL10* mRNA expression ($p=0.007$). X and Y axises show log 2 fold change of gene expression

Table 3 Association between *PYGO2* and *IL10* expression in ESCC patients

Expression pattern		IL10		*P* value
		Overexpression	Normal/under expression	
PYGO2	Overexpression	14	4	**0.023**
	Normal/under expression	16	24	

samples with moderately differentiated tumors whereas in patients with well and poorly differentiated tumors, this percentage was 1 of 12 (8.3%) and 4 of 11 (36.4%), respectively. *PYGO2* and *IL10* expression were lowest in well-differentiated tumors compared to moderately differentiated tumors. Besides, tumor samples from the middle region of esophagus expressed *IL10* at higher levels than lower region tumors. There were no significant correlations between *PYGO2* and *IL10* expression and other clinicopathological variables.

Forced expression of *PYGO2* changed *IL10* expression

After the transfection of KYSE-30 and YM1 cells, a functional study was performed to evaluate the expression of *PYGO2* and *IL10*. *PYGO2* was overexpressed in KYSE-30 and YM1 cells compared to controls, with log 2 fold changes nearly 6.5 and 4.8, respectively. This level of *PYGO2* overexpression caused a significant increase in *IL10* gene expression nearly 4 and 2.5 in KYSE-30 and YM1 cells, respectively.

Discussion

The Wnt/β-catenin signaling pathway, as an evolutionarily-conserved pathway, employs both proinflammatory and anti-inflammatory functions [12]. Its activation is associated with deficiency of an immune infiltrate in cancer microenvironment [13]. Deregulation of this pathway was detected in liver, osteosarcoma, breast, colorectal, gastric, cervical, and bone cancers [14]. Consist with these findings, several reports corroborate activation of this pathway in ESCC.

Here we manifested that mRNA expression of *PYGO2* and *IL10*, as two important genes involved in Wnt signaling pathway, was upregulated in ESCC and intriguingly, there was a significant correlation between these genes which probably occur through Wnt/β-catenin signaling pathway. Having performed functional study, we demonstrated that enforced expression of *PYGO2* changed *IL10* expression in ESCC cells. Furthermore, we found that concomitant expression of the genes was strongly correlated with clinical features of ESCC patients and different indices of poor prognosis which may introduce their concomitant expression as a new regulatory axis in ESCC.

PYGO2 is an essential transcriptional coactivator of the Wnt/β-catenin signaling pathway which promotes β-catenin-LEF/TCF transcriptional activation through adapter protein BCL9 [15]. It contributes in expression of highly transcribed RNAs during cell-cycle progression and DNA replication, growth and expansion of cancer cells, as well as histone code interpretation through β-catenin– histone methyltransferase/histone acetyltransferase (HMT/HAT) association [16]. Furthermore, it is applied in a MYC-dependent transcriptional program as an epigenetic accessory protein to regulate cell division [17]. Up-regulated expression of *PYGO2* has been illustrated in diverse malignant tumors including ovarian, colon, liver, and cervical cancers. In addition, its oncogenic role is confirmed in hepatic carcinoma, prostate adenocarcinoma, lung and breast cancers as well as renal cell carcinoma and glioma [18–21]. In line with these reports, *PYGO2* overexpression has been reported in ESCC in association with the grade of tumor cell differentiation [22]. Intriguingly, we also observed high level expression of *PYGO2* in 31.0% of ESCC samples. It has been revealed that *PYGO2* overexpression promotes lymph node metastasis in lung and prostate cancers [23]. Here we found that 7 of 18 *PYGO2* overexpressed tumors, were metastasized to the lymph nodes, probably reflecting the importance of *PYGO2* in metastasis process. Likewise, *PYGO2* activates Wnt target genes such as *AXIN1, cyclin D1*, and *IL10*. Surprisingly, we found a significant correlation between *PYGO2* and *IL10* expression. In tumors with high levels of *PYGO2, IL10* expression was significantly higher compared to those with normal *PYGO2* expression. In addition, induced expression

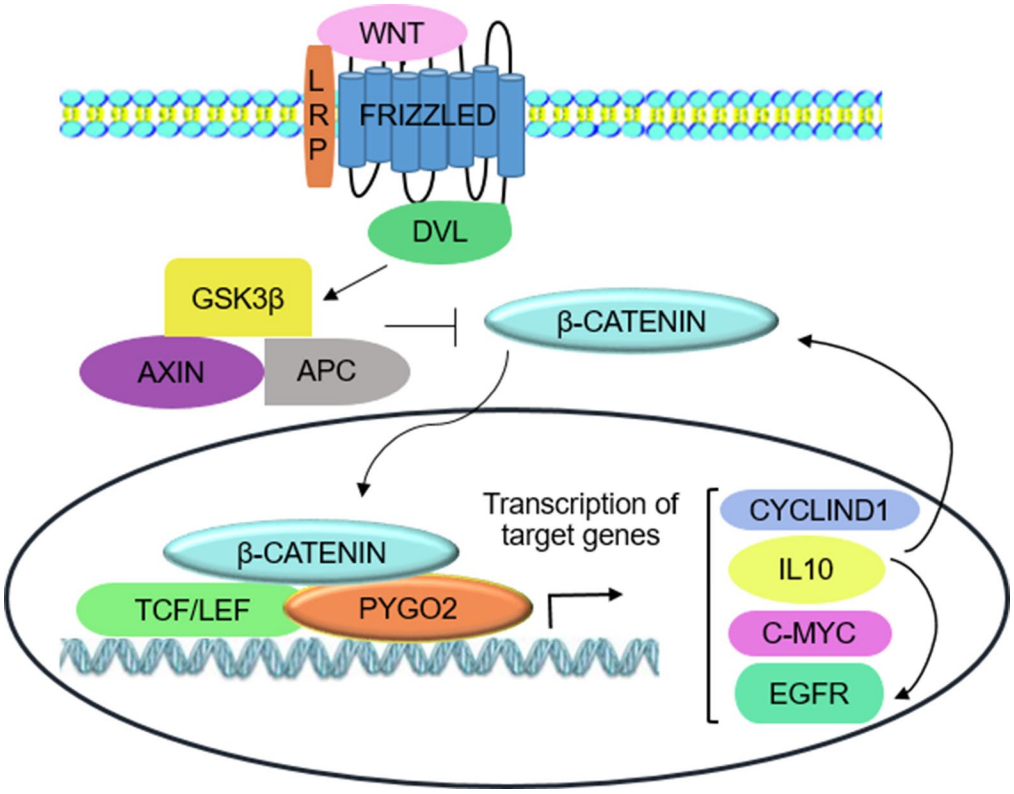

Fig. 4 Potential correlation between *PYGO2* and *IL10* expression through the Wnt/β-catenin signaling pathway in ESCC progression. Binding of Wnt ligand to *FZD* and *LRP* induces inactivation of the *AXIN-APC-GSK3β* destruction complex via *DVL*, leading to *β-catenin* accumulation and transition to the nucleus. Then, *PYGO2* as a coactivator of the transcriptional complex (*LEF/TCF/β-catenin*), may directly activate transcription of target genes such as *IL10*. Furthermore, *IL10* expression manifests direct correlation with *FZD, WNT, LRP* expression, *β-catenin* accumulation, and augmentation of *EGFR* levels in this pathway

of *PYGO2* in ESCC lines increased expression of *IL10* significantly. This finding is noteworthy, suggesting the regulatory role of *PYGO2* as a transcriptional activator of *IL10* expression probably through Wnt/β-catenin signaling pathway (Fig. 4). Since co-overexpression of *PYGO2* and *IL10* was significantly correlated with different indices of poor prognosis in ESCC, Wnt/β-catenin signaling may be involved in ESCC tumorigenesis through these genes.

Cytokines play critical roles in cancer, inducing tumor metastasis and invasion, as well as apoptosis inhibition [24]. Interleukin-10 is a pleiotropic cytokine with broad anti-inflammatory properties through suppression of both dendritic cell and macrophage function. It suppresses the antitumor immune response, so it is indispensable for tumor development [25]. *IL10* has widely been examined in various types of human malignancy. Elevated serum concentrations of *IL10* is related with poor prognosis and adverse disease stage in gastric, colon, lung, bone sarcoma, hepatocellular carcinoma, melanoma, and pancreatic malignancies. It is interesting that our findings disclosed *IL10* overexpression in 51.7% of ESCC samples which is consisting with previous reports in ESCC [26, 27]. Furthermore, we observed 25 of the 30 *IL10* overexpressed ESCCs (83.33%) were invaded to the esophagus adventitia presenting T3 depth of tumor invasion. This data corroborates recent reports in melanoma and cervical cancer [28, 29]. According to *IL10* function in tumor development, it may be assumed that IL10 induce tumor invasion and progression by suppressing immune responses and stimulating angiogenesis.

IL10 has been illustrated to contribute in divers signaling pathways. It suppresses starvation induced autophagy in HS-derived fibroblasts (HSFs) through cross talk between the *IL10*/AKT-mTOR and *IL10*/*IL10R*-STAT3 pathways [30]. Moreover, Notch signaling can induce *IL10* expression through TH1 cells which involves STAT4-dependent process [31]. This gene is closely linked with various Wnt pathway components, wherein multiple Wnt ligands regulate its responses [32]. In addition, fibroblast-derived *Wnt16B* induces *IL10* secretion in dendritic cells. *IL10* expression is associated with the levels of *EGFR* (as a Wnt target gene) and *β-catenin* accumulation in lung cancer and melanoma, respectively. Similarly, *IL10* level is directly correlated with the level of *FZD9* and *LRP5/6* in Wnt pathway [33–35]. Therefore, all these evidences support the involvement of *IL10* in Wnt pathway active state.

The concomitant expression of *PYGO2* and *IL10* in this study was significantly correlated with depth of tumor invasion. Tumor invasion is a predictor of lymph node metastasis in several cancers such as ESCC. Interestingly, of 25 patients with lymph node metastasis, 6 (24.0%) co-overexpressed of both genes, while in 75.8% of patients (25 of 33) without lymph node metastasis, *PYGO2* and *IL10* were not co-overexpressed. Having consider these results, the correlation between the genes may be envisaged potentially to activate a cascade leading to malignant invasion and metastasis. In the case of tumor stage, the co-overexpression of the genes was significantly correlated with advanced stage of tumor progression (stage III). It was largely expected, since stage III is associated to lymph node involvement. This result confirms the similar findings in NSCLC, where *IL10* overexpression was correlated with high degree of stage and lymph node metastases [36]. Considering this fact that ESCC initiation and progression is a multistep process with poor prognosis, the potential correlation between *PYGO2* and *IL10* may be considered as an efficient prognostic panel of markers in ESCC patients.

Previous studies have separately considered *PYGO2* and *IL10* as co-transcription factor and target gene in Wnt pathway, respectively. Subsequently, the involvement of deregulated Wnt/β-catenin pathway has been revealed in ESCC carcinogenesis and progression. Based on these reports and our results, it seems likely that *PYGO2* may activate *IL10* expression through Wnt/β-catenin signaling pathway in ESCC which may augment ESCC carcinogenesis and aggressiveness.

Conclusion

To sum up, our work disclosed the concomitant expression of *PYGO2* and *IL10* in ESCC patients and regulatory role of *PYGO2* in *IL10* gene expression in ESCC cells. We indicated an association between these genes probably through Wnt/β-catenin signaling pathway in ESCC. Furthermore, *PYGO2* and *IL10* co-overexpression was found in significant correlation with tumor poor prognosis including tumor depth of invasion, lymph nodes metastasis, surgical stage, and grade of tumor differentiation. Therefore, it can be introduced as a new regulatory axis for ESCC poor prognosis. To the best of our knowledge, this is the first report found this correlation in ESCC to date. Considering the involvement of *IL10* as an anti-inflammatory cytokine and *PYGO2* role in elevated tumor invasion and metastasis, possible functional interaction between these factors may promote a process which induces malignant metastasis and invasion in ESCC.

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Author contributions
F.A.Sh., N.H. and F.N. performed the experiments and drafted the manuscript. M.A.A.Sh. was involved in data analysis and drafting. MMF designed the study, analyzed data, and edited the manuscript.

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Data availability
All raw data are available on case of reasonable request from corresponding author.

Declarations

Ethics approval and consent to participate
The study was approved by ethics committee of Mashhad University of Medical Sciences (No. 88098) according to the Ethical Helsinki Declaration agreement and informed consent was obtained from all individual participants included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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