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Araştırma Makalesi

Apoptosis related PDCD4: Promising Novel Biomarker Early Detection of Oral Cancer

Apoptoz İlişkili PDCD4: Oral Kanserin Erken Tanısında Umut Vaad Edici Yeni Bir Biyobelirteç

Leyla Bozdağ[1], Leyla Açık[2], Halil Erhan Ersoy[3], Ömer Bayır[4], Mehmet Hakan Korkmaz[5], Nur Mollaoğlu[6], Sibel Elif Gültekin[7]

ABSTRACT

Aim: This study aimed to investigate the potential role of the PDCD4 gene, involved in the progression of the oral potentially malign disorder (OPMD) and oral squamous cell carcinoma cancer (OSCC).

Material and Methods: The study was conducted on saliva and blood samples that were collected from OSCC (n=6), OPMD (n=6) patients, and healthy (n=6) individuals. The expression of PDCD4 was determined by using qRT-PCR. Kruskal Wallis test was performed to estimate the relationship between PDCD4 expression and clinical characteristics of OSCC and OPMD patients. The correlation between PDCD4 expression and patients groups was tested with the Rank Spearman test.

Results: PDCD4 mRNA expression levels were assessed in 6 OSCCs, 6 OPMD patients, and 6 healthy individuals saliva and blood. In both blood and saliva, PDCD4 mRNA expression levels were 6/6 (100%) underexpressed in OSCC, while it was underexpressed in 5/6 (83%) of OPMD. The mean value of PDCD4 was significantly downregulated in OPMD and OSCC than in healthy ($p<0.05$). The Spearman rank test obtained a p-value for the 2 tail test of 0.024 ($p < 0.05$), which showed a significant correlation between both, while the correlation coefficient of -0.530 showed a strong negative correlation of PDCD4 downregülation and OPMD, OSCC.

Conclusions: PDCD4 expression level is correlated with OSCC and OPMD. This study indicates that PDCD4 expression levels in liquid biopsies may have potential as a diagnostic biomarker in OSCC and OPMD.

ÖZET

Amaç: Bu çalışma, oral potansiyel malign bozukluk (OPMD) ve oral skuamöz hücreli karsinom karsinomun (OSCC) ilerlemesinde rol oynayan PDCD4 geninin potansiyel rolünü araştırmayı amaçlamıştır.

Gereç ve Yöntem: Çalışma OSCC (n=6), OPMD (n=6) ve sağlıklı (n=6) bireylerden alınan tükürük ve kan örnekleri üzerinde gerçekleştirildi. PDCD4'ün ifadesi, qRT-PCR kullanılarak belirlendi. PDCD4 ekspresyonu ile OSCC ve OPMD hastalarının klinik özellikleri arasındaki ilişkiyi tahmin etmek için Kruskal Wallis testi yapıldı. PDCD4 ekspresyonu ile hasta gruplarının korelasyonu Rank Spearman testi ile test edildi.

Bulgular: PDCD4 mRNA ekspresyon seviyeleri 6 OSCC'de, 6 OPMD hastasında ve 6 sağlıklı bireyde tükürük ve kanda değerlendirildi. Hem kanda hem de tükürükte, PDCD4 mRNA ekspresyon seviyeleri OSCC'de 6/6 (%100) düşük ifade edilirken, OPMD'nin 5/6'sında (%83) düşük ifade edildi. Ortalama PDCD4 değeri, OPMD ve OSCC'de sağlıklı olandan önemli ölçüde düşük ifade edildi ($p<0.05$). Rank Spearman testi ile $p= 0.024$ ($p < 0.05$) OSCC ve OPMD arasında anlamlı bir korelasyon gösterirken; -0.530 korelasyon katsayısı, , PDCD4 'ün OPMD ve OSCC arasında güçlü bir negatif korelasyona sahip olduğunu gösterdi.

Sonuç: PDCD4 ekspresyon seviyesi, OSCC ve OPMD ile ilişkilidir. Bu çalışma, sıvı biyopsilerdeki PDCD4 ekspresyon seviyelerinin OSCC ve OPMD'de tanısal bir biyobelirteç olma potansiyeline sahip olabileceğini göstermektedir.

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İletişim: Arş. Gör. Leyla Bozdağ
Gazi Üniversitesi Diş Hekimliği Fakültesi, Bişkek Cad. 1.sok. No: 4 Çankaya/Ankara
E-posta: leyla.arslan97@gmail.com
[1] PhD student, Gazi University, Faculty of Science, Department of Biology, Ankara, Türkiye
[2] Prof. Dr., Gazi University, Faculty of Science, Department of Biology, Ankara, Türkiye
[3] PhD student, Gazi University, Faculty of Dentistry,Department of Oral and Maxillofacial Surgery, Ankara, Türkiye
[4] Doç. Dr., Department of Otorhinolaryngology, University of Health Sciences Dışkapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey
[5] Prof. Dr., Department of Otorhinolaryngology, University of Health Sciences Dışkapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey
[6] Prof. Dr., Gazi University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Ankara, Türkiye
[7] Prof. Dr., Gazi University, Faculty of Dentistry, Department of Basic Sciences, Department of Oral Pathology, Ankara, Türkiye

INTRODUCTION

Oral squamous cell carcinoma (OSCC), also known as oral cancer, is the sixth most common cancer globally. According to GLOBOCAN 2020 data, there are 377.713 new cases worldwide, whilst, 2.103 new cases, with 592 deaths are reported in Turkey.[1] Oral cancer (OSCC) may arise de novo or from oral potentially malignant disorders (OPMD) such as leukoplakia, erythroplakia, and erythroleukoplakia. The rate of dysplastic changes in OPMD is closely associated with progression to invasiveness. Despite advances in molecular studies, histologically dysplasia grading is still the unique prognostic parameter that indicates the transition to oral cancer.

Carcinogenesis is a multistep process involving the accumulation of numerous genetic and epigenetic changes, such as oncogene activation and tumor suppressor gene inactivation, at the molecular level. Despite the advances in the diagnosis and treatment of this lethal disease, the survival rate is just 50%, owing to patients late presentation to doctors and surgeons. Early detection and intervention are critical in light of the expanding illness load. Various approaches, ranging from simple chair-side investigations such as vital staining to complex laboratory procedures, have constituted the spectrum of diagnostic aids in the early identification of oral precancer and cancer, starting with good clinical examination. However, due to the late identification of oral cancer, complex diagnostic tools such as lab-on-a-chip, microfluidics, nano-diagnostics, liquid biopsy, and omics technology such as genomics, transcriptomics, proteomics, and metabolomics are required.[2] Obtaining successive tumor biopsies in advanced OSCC may be difficult. As a result, conducting a liquid biopsy to discover possible treatment targets is an intriguing alternative to tumor biopsy. Several research has looked into the viability of detecting somatic changes in OSCC utilizing liquid biopsy.[3-7]

The expression of PDCD4 has been reported to decrease in a variety of human cancers[8-11], including the stomach, pancreas, colon, lung, prostate, ovary and liver. However, the molecular mechanism of the decrease of PDCD4 expression in human malignancies is yet unknown.[8]

In this study, we aimed to analyze the expression pattern of the PDCD4 gene in blood and saliva samples of OSCC and OPMD patients as a potential noninvasive diagnostic test for early detection of oral squamous cell carcinoma.

MATERIAL AND METHODS

Ethics statement

Before the trial, all participants signed an informed consent form. The Ethics Committee of Clinical Experiment of Gazi University Dentistry Faculty accepted the collection and use of all blood and saliva samples, stating that they followed all relevant ethical criteria on October 19, 2020 (approval no. E.28270).

Patients and clinical data

A total of 12 patients (OSCC n=6, OPMD n=6) and 6 healthy individuals were attended at the Department of Oral and Maxillofacial Surgery, Department of Otorhinolaryngology-Head and Neck Surgery, University of Health Sciences, Ankara Diskapi Training and Research Hospital (Ankara Turkey). The clinical and histological photographs of the OSCC are shown in Figure 1. Histological examination was performed at 40X magnification in tissue samples stained with hematoxylin and eosin.

The inclusion criteria for OSCC patients are as follows:

1) Presence of a primary tumor diagnosed as squamous cell carcinoma located in the 2/3 anterior part of the tongue, the floor of the mouth, buccal mucosa, gingiva, retromolar region, hard palate, 2) Absence of Human Papillomavirus (viral) etiology in tumor development, 3) Recurrence and absence of any other malignancy, 4) Diagnosis made by incisional biopsy.

The OPMD group consisted of individuals with oral leukoplakia-erythroplakia clinical pre-diagnosis and histologically diagnosed as dysplasia with the oral mucosal lesion. This group (n=6 people) was evaluated according to the binary system[12] and diagnosed with low-grade dysplasia.

Healthy volunteers who have undergone impacted 3 rd molar surgery served as a control group. Demographic information of the patients was recorded and shown in Table 1.

Table 1. Demographic data of the OSCC and OPMD patients and healthy individuals

	OSCC group (n=6)	OPMD group (n=6)	Controlgroup (n=6)	P value
Age[a]				
≤44	2	3	6	**.001**
>44	4	3		
Sex				.081
Male	3	3	5	
Female	3	3	1	
Smoking				.492
Yes	5	4	3	
No	1	2	3	
Alcohol				.492
Yes	3	4	3	
No	3	2	3	
Tumor location[b]				.329
Tongue	3			
Alveolarmucosa	1	2		
Buccal mucosa	1	2		
Retromolar mucosa	1	2		
OPMD Histological classification (Binary system)				
High grade dysplasia				
Low grade dysplasia		6		
Surveillance				1.000
Alive with disease	6	6		

[a]Patients were categorized into groups based on the median age.
[b]Tumor localization has been demonstrated from the outset for diagnostic purposes

Collection of saliva and blood

A total of 18 blood and 18 saliva samples were collected from OSCC and OPMD patients and healthy individuals, n=3 blood and n=3 saliva (of OSCC patients) taken from the Department of Otorhinolaryngology-Head and Neck Surgery, University of Health Sciences, Ankara Diskapi Training and Research Hospital (Ankara Turkey).

The same protocol as described by Navazesh et al. was performed for the saliva collection using standard technics.[13] Five milliliters of unstimulated saliva samples were collected into a 50-milliliter sterile falcon which, was homogenized for 20 seconds by vortexing, followed by centrifugation at 2.600 g for 15 minutes at 4°C. The supernatant was collected and stored in an eppendorf tube. Labeled samples were lifted to -80°C.

The blood that would be isolated was collected in containers that would not allow the cells' characteristics to be lost (EDTA tube). The blood was stored at -80°C after being centrifuged at various speeds, including 1.000 g at 10 minutes 4°C.

RNA isolation and cDNA synthesis

Total RNA was isolated from blood and saliva using the Paxgene Blood miRNA İsolation Kit (Qiagen, Germany) and the miRNeasy mini kit (Qiagen, Germany), respectively, following the manufacturer's recommendations. 1 g of total RNA was reverse transcribed into cDNA using the Transcriptor High Fidelity cDNA Synthesis kit (Roche Diagnostics, Mannheim, Germany) under the following conditions to assess PDCD4 mRNA expression: 30 minutes at 55°C, 5 minutes at 85°C. Then, using the RT product, Quantinova LNA Probe PCR Assay (Qiagen, Germany), and specific primers for PDCD4 and ActB, real-time PCR was performed.

RT-qPCR

On an Applied Biosystems 480 LightCycler Detection System, real-time PCR was done using a Quantinova Probe PCR Kit (Applied Biosystems, Germany). The reactions were incubated at 95°C for 10 minutes in a 96-well optical plate, followed by 45 cycles of 95°C for 15 seconds and 60°C for 60 seconds. All of the reactions were carried out three times. The cycle threshold (CT) data were determined after the reaction using fixed threshold settings, and the mean CT was calculated from the triplicate PCRs. To compare each condition to the controls, a comparative CT approach was applied. The amount of mRNA in comparison to the internal control ActB was estimated using the formula $2^{-\Delta\Delta CT}$, in which $\Delta\Delta CT = (CT_{PDCD4} - CT_{ActB})$ tumor- $(CT_{PDCD4} - CT_{ActB})$ control.

Statistical analysis

To compare the PDCD4 expression in OSCC, OPMD and the healthy patient were tested with the Kruskal Wallis test. Spearman correlation was performed to examine the association between the expression status of PDCD4 mRNA and patient groups. P<0.05 was considered to indicate a statistically significant difference.

RESULT

Clinical characteristics

As shown in Table 1, statistically significant differences were found in the age of the OSCC patients when compared to OPMD patients and the control group (p=0.001). The mean age of the OSCC patients was 59.3 whereas, it was 42 for OPMD and 20.6 for healthy individuals. The most common anatomical site was the tongue for the OSCC, whilst equal distribution was seen in OPMD patients. In this study, there were not any significant differences between the groups in the context of the clinical parameters.

PDCD4 downregulated in OPMD and OSCC

We analyzed that the PDCD4 expression levels both in blood and saliva samples were significantly lower in patients with OSCC and OPMD groups than in healthy individuals (p <0.05) (Figure 1).

When the relative expression level of PDCD4 mRNA in blood and saliva samples of patients with OSCC was examined, both were found to be downregulated. However, 8.3% less PDCD4 mRNA expression was found in the saliva sample compared to the blood sample (Figure 4).

Table 2. Correlation between PDCD4 expression and OSCC, OPMD patients

	Patient Groups	PDCD4 expression
Patient Groups Spearman Correlation	1.000	-.530*
Sig. (2-tailed)		.024
N	18	18
PDCD4 expression Spearman Correlation	-.530*	1.000
Sig. (2 tailed)	.024	
N	18	18

*Correlation is significant at the 0.05 level (2-tailed).

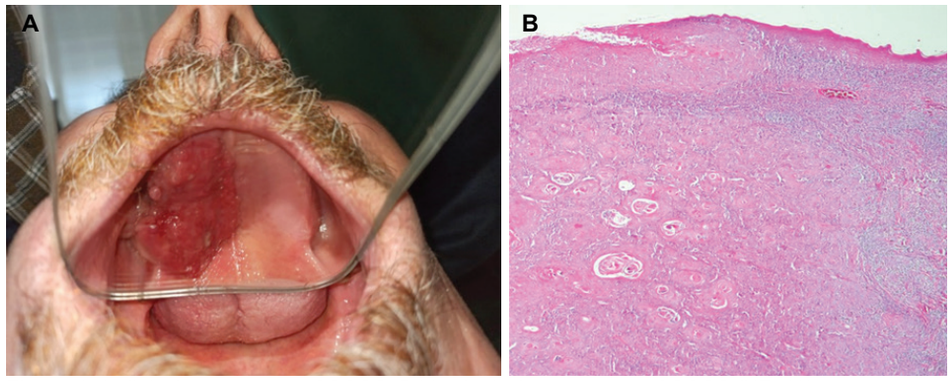

Figure 1. A clinical appearance of OSCC, B histological appearance of OSCC (HEx40).

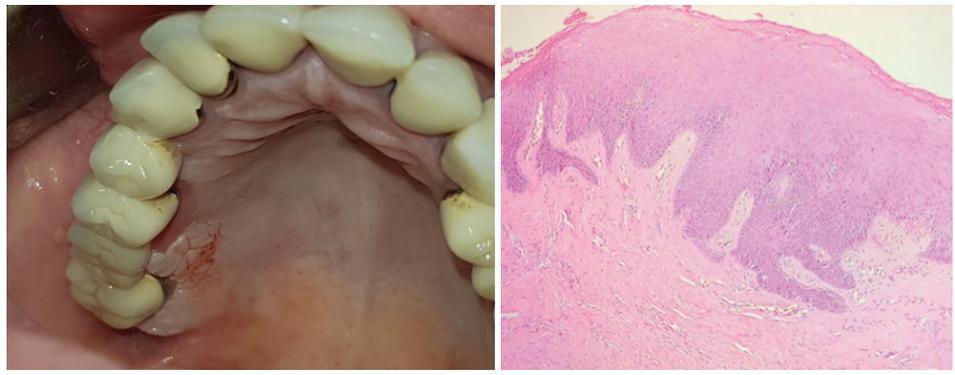

Figure 2. A clinical appearance of OPMD, B histological appearance of low-grade oral dysplasia (HEx40).

When the relative expression level of PDCD4 mRNA in blood and saliva samples of patients with OPMD was examined, PDCD4 expression was found to be downregulated in both samples. However, PDCD4 mRNA expression was 500 times lesser in the blood than in the saliva samples (Figure 4).

A statistically significant correlation was only found between age and PDCD4 mRNA expression levels in terms of clinical data ($p<0.01$).

A correlation test with the Spearman rank was obtained to find out the correlation of PDCD4 expression with an incidence of OPMD and OSCC. We found a significant correlation between PDCD4 expression and OPMD, OSCC ($p= 0.024$). This test shows a strong negative correlation between PDCD4 expression and patient groups (correlation coefficient, - 0.530) (Table 2).

DISCUSSION

PDCD4 was first identified as a tumor suppressor, and mice lacking the protein develop spontaneous lymphomas.[14] PDCD4 had a role in tumor growth by regulating cell death, proliferation, invasion, and metastasis. It has been proposed as a potential biomarker for the prognosis and diagnosis of various cancers.

Despite recent advances in the molecular studies of OSCC, the biomarker for both the early diagnosis and prognosis of OSCC has not been available yet.

Although there are several studies conducted on the tissue specimens and cell lines of various cancers including lung[15], OSCC[16,20], colon[17, 18], ovary[19] which determined the PDCD4 expression levels whereas there is only one study on esophageal cancer that has been studied with PDCD4 in plasma.[21] There is a lack of research on OSCC and OPMD which are performed with the liquid biopsies. No study was found with PDCD4 in blood and saliva samples in OSCC and OPMD.

To our knowledge, the current study presents the first data concerning the PDCD4 mRNA levels in saliva and blood samples in OSCC and OPMD patients. Liao et al. reported that PDCD4 mRNA expression level was decreased in plasma samples obtained from patients with esophageal cancer compared to healthy controls.[22] Liu et al. reported that in tongue squamous cell carcinoma tissue specimen PDCD4 mRNA expression was downregulated.[23]

According to the study of Chen et al. in adenocarcinomas, PDCD4 mRNA expression was decreased. This was most apparent in high-grade tumors, which showed very significantly decreased PDCD4 expression compared with low-grade tumors.[15]Other studies show that PDCD4 inhibited colon cancer cell invasion and intravasation, indicating PDCD4 is a regulator of invasion and metastasis.[24]

Our study results showed that PDCD4 was under-expressed at the mRNA levels in both saliva and blood. Similar findings that we did was reported in the tumor tissue specimens of OSCC.[20] In the study conducted by Reis et al.[20] in OSCC tissue samples, loss of mRNA expression of PDCD4 showed that PDCD4 may be a prognostic marker in OSCC. Similarly, in our study, PDCD4 mRNA expression level was found to be decreased in blood and saliva samples. İn this case, PDCD4 can be used as a prognostic biomarker in blood and saliva samples from OSCC patients.

According to Ludwig et. al. gastrointestinal dysplasias in histology doubtful cases (differential diagnosis between regenerative and dysplastic lesions) such as ulcerative colitis and Crohn's colitis PDCD4 mRNA expression levels downregulated.[25] In our studies with OPMD patients in both saliva and blood samples, PDCD4 mRNA expression levels were downregulated.

Initially associated with apoptosis, the nuclear protein PDCD4 was quickly reclassified as a tumor suppressor gene and prospective target for anti-cancer therapy. Indeed, an increasing number of evidence supports PDCD4 expression as suppressing cancer cell invasion and metastasis.[26]

Recent studies demonstrated that PDCD4 is involved in transcription, translation of proteins involved in neoplastic transformation, such as eukaryotic initiation factors (eIFs) [27], and cell signaling pathways.[26] PDCD4 regulates translation by interacting with the eIF4A and eIF4G1 translation initiation factors [28, 29] PDCD4 interacts directly with eIF4A via its MA3-c domain reducing eIF4A helicase activity and disrupting the assembly of the eIF4F complex, causing cap-dependent translation to be interrupted and cell transformation to be inhibited. [30]

In both saliva and blood samples from patients with malignant oral malignancies and oral potential malignant diseases, we found significant PDCD4 under-expression/loss. The fact that decreased PDCD4 expression in metastatic OSCCs is associated with shorter survival and disease-free survival in OSCC patients implies that PDCD4 could be a clinically useful biomarker with prognostic significance. Furthermore, decreased PDCD4 expression may be a therapeutically meaningful biomarker with predictive value in individuals with oral potentially malignant lesions.

Understanding PDCD4 expression patterns, regulation, and role in OSCC and OPMD may be useful for investigating PDCD4 as a possible therapeutic target in OSCC.

CONCLUSION

The PDCD4 mRNA gene expression level in the non-invasive blood and saliva materials we used in our study showed similar results with the PDCD4 mRNA gene expression level in the invasive tissue use we encountered in the literature. It has been suggested that PDCD4 expression level could be a useful material and biomarker in oral squamous cell carcinoma.

Similarly, this study found that low PDCD4 mRNA expression in the blood and saliva of patients with oral potentially malignant lesions increases the likelihood of oral squamous cell carcinoma and that PDCD4 is a viable biomarker for early identification due to its availability in blood and saliva.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.

2. Madhura MG, Rao RS, Patil S, Fageeh HN, Alhazmi A, Awan KH. Advanced diagnostic aids for oral cancer. Dis Mon 2020;66:101034.

3. Wang J, Chang S, Li G, Sun Y. Application of liquid biopsy in precision medicine: opportunities and challenges. Front Med 2017;11:522-7.

4. van Ginkel JH, Huibers MMH, van Es RJJ, de Bree R, Willems SM. Droplet digital PCR for detection and quantification of circulating tumor DNA in plasma of head and neck cancer patients. BMC Cancer. 2017;17(1):428.

5. Braig F, Voigtlaender M, Schieferdecker A, Busch CJ, Laban S, Grob T, et al. Liquid biopsy monitoring uncovers acquired RAS-mediated resistance to cetuximab in a substantial proportion of patients with head and neck squamous cell carcinoma. Oncotarget 2016;7:42988-95.

6. Perdomo S, Avogbe PH, Foll M, Abedi-Ardekani B, Facciolla VL, Anantharaman D, et al. Circulating tumor DNA detection in head and neck cancer: evaluation of two different detection approaches. Oncotarget 2017;8:72621-32.

7. Mazurek AM, Rutkowski T, Fiszer-Kierzkowska A, Malusecka E, Skladowski K. Assessment of the total cfDNA and HPV16/18 detection in plasma samples of head and neck squamous cell carcinoma patients. Oral Oncol 2016;54:36-41.

8. Guo PT, Yang D, Sun Z, Xu HM. PDCD4 functions as a suppressor for pT2a and pT2b stage gastric cancer. Oncol Rep 2013;29:1007-12.

9. Jajoo S, Mukherjea D, Kaur T, Sheehan KE, Sheth S, Borse V, et

al. Essential role of NADPH oxidase-dependent reactive oxygen species generation in regulating microRNA-21 expression and function in prostate cancer. Antioxid Redox Signal 2013;19:1863-76.

10. Ma G, Zhang H, Dong M, Zheng X, Ozaki I, Matsuhashi S, et al. Downregulation of programmed cell death 4 (PDCD4) in tumorigenesis and progression of human digestive tract cancers. Tumour Biol 2013;34:3879-85.

11. Zhen Y, Li D, Li W, Yao W, Wu A, Huang J, et al. Reduced PDCD4 Expression Promotes Cell Growth Through PI3K/Akt Signaling in Non-Small Cell Lung Cancer. Oncol Res 2016;61-8.

12. Kujan O, Khattab A, Oliver RJ, Roberts SA, Thakker N, Sloan P. Why oral histopathology suffers inter-observer variability on grading oral epithelial dysplasia: an attempt to understand the sources of variation. Oral Oncol 2007;43:224-31.

13. Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci 1993;694:72-7.

14. Hilliard A, Hilliard B, Zheng SJ, Sun H, Miwa T, Song W, et al. Translational regulation of autoimmune inflammation and lymphoma genesis by programmed cell death 4. J Immunol 2006;177:8095-102.

15. Chen Y, Knosel T, Kristiansen G, Pietas A, Garber ME, Matsuhashi S, et al. Loss of PDCD4 expression in human lung cancer correlates with tumour progression and prognosis. J Pathol 2003;200:640-6.

16. Cervigne NK, Reis PP, Machado J, Sadikovic B, Bradley G, Galloni NN, et al. Identification of a microRNA signature associated with progression of leukoplakia to oral carcinoma. Hum Mol Genet. 2009;18(24):4818-29.

17. Mudduluru G, Medved F, Grobholz R, Jost C, Gruber A, Leupold JH, et al. Loss of programmed cell death 4 expression marks adenoma-carcinoma transition, correlates inversely with phosphorylated protein kinase B, and is an independent prognostic factor in resected colorectal cancer. Cancer 2007;110:1697-707.

18. Wang Q, Sun Z, Yang HS. Downregulation of tumor suppressor Pdcd4 promotes invasion and activates both beta-catenin/Tcf and AP-1-dependent transcription in colon carcinoma cells. Oncogene. 2008;27:1527-35.

19. Wei NA, Liu SS, Leung TH, Tam KF, Liao XY, Cheung AN, et al. Loss of Programmed cell death 4 (Pdcd4) associates with the progression of ovarian cancer. Mol Cancer 2009;8:70.

20. Reis PP, Tomenson M, Cervigne NK, Machado J, Jurisica I, Pintilie M, et al. Programmed cell death 4 loss increases tumor cell invasion and is regulated by miR-21 in oral squamous cell carcinoma. Mol Cancer 2010;9:238.

21. Hiyoshi Y, Kamohara H, Karashima R, Sato N, Imamura Y, Nagai Y, et al. MicroRNA-21 regulates the proliferation and invasion in esophageal squamous cell carcinoma. Clin Cancer Res 2009;15:1915-22.

22. Liao J, Liu R, Shi YJ, Yin LH, Pu YP. Exosome-shuttling microRNA-21 promotes cell migration and invasion-targeting PDCD4 in esophageal cancer. Int J Oncol 2016;48:2567-79.

23. Liu C, Tong Z, Tan J, Xin Z, Wang Z, Tian L. MicroRNA-21-5p targeting PDCD4 suppresses apoptosis via regulating the PI3K/AKT/FOXO1 signaling pathway in tongue squamous cell carcinoma. Exp Ther Med 2019;18:3543-51.

24. Asangani IA, Rasheed SA, Nikolova DA, Leupold JH, Colburn NH, Post S, et al. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene 2008;27:2128-36.

25. Ludwig K, Fassan M, Mescoli C, Pizzi M, Balistreri M, Albertoni L, et al. PDCD4/miR-21 dysregulation in inflammatory bowel disease-associated carcinogenesis. Virchows Arch 2013;462:57-63.

26. Lankat-Buttgereit B, Goke R. The tumour suppressor Pdcd4: recent advances in the elucidation of function and regulation. Biol Cell 2009;101(6):309-17.

27. LaRonde-LeBlanc N, Santhanam AN, Baker AR, Wlodawer A, Colburn NH. Structural basis for inhibition of translation by the tumor suppressor Pdcd4. Mol Cell Biol 2007;27:147-56.

28. Kang MJ, Ahn HS, Lee JY, Matsuhashi S, Park WY. Up-regulation of PDCD4 in senescent human diploid fibroblasts. Biochem Biophys Res Commun 2002;293:617-21.

29. Yang HS, Jansen AP, Komar AA, Zheng X, Merrick WC, Costes S, et al. The transformation suppressor Pdcd4 is a novel eukaryotic translation initiation factor 4A binding protein that inhibits translation. Mol Cell Biol 2003;23:26-37.