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New Proteins in the Differentiation of Papillary Renal Cell Carcinoma From Clear Cell Renal Cell Carcinomas; Importance of DARS2, Reelin and Enkurin

Papiller Renal Hücreli Karsinomun Berrak Hücreli Renal Hücreli Karsinomlardan Ayrımında Yeni Proteinler; DARS2, Reelin ve Enkurin'in Önemi

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Abstract

Aim: The purpose of the present study was to examine the roles of new proteins DARS2, Reelin, and Encurin in the differentiation of Clear-Cell Renal Cell Carcinoma (RCC) and Papillary Renal Cell Carcinoma (RCC). Clear-cell RCC is the most common malignancy of the kidney, and papillary RCC is the second most common malignant malignancy in this respect. They are neoplasms and show similarity to each other, both histologically and morphologically, in some cases. Differential diagnosis is important because treatment approaches and prognoses are different. Although careful histopathological examination and specific immunohistochemical markers are important for diagnosis, there are no specific antibodies that can be used reliably and the search for biomarkers continues in this regard.

Material and Method: A total of 30 Clear-Cell RCC and 30 Papillary RCC cases were included in the present study. Patients were identified retrospectively by reviewing the hospital database and pathological reports. Pathological data were obtained from hospital medical archives and pathology reports.

Results: It was found that DARS2, Reelin, and Encurin proteins were significantly higher in papillary RCC when compared to clear-cell RCC.

Conclusion: It was concluded that DARS2, Reelin, and Encurin proteins may be potential biomarkers for the differentiation of Papillary RCC and Clear-Cell RCC.

Keywords: DARS, Reelin, Encurin, papillary renal cell carcinoma, clear-cell renal cell carcinoma, biomarker, immunohistochemistry

Öz

Amaç: Bu çalışma ile berrak hücreli böbrek hücreli karsinomlardan (BHK), papiller böbrek hücreli karsinom (BHK)'un ayırmında yeni proteinler olan DARS2, reelin ve enkurinin rollerinin incelenmesi amaçlandı. Berrak hücreli BHK, böbreğin en yaygın, papiller BHK ise ikinci sıklıkta görülen malign neoplazmları olup bazı durumlarda histolojik ve morfolojik olarak birbirleri ile benzerlik göstermektedir. Tedavi yaklaşımları ve prognozları farklı olduğu için ayırıcı tanıları önemlidir. Dikkatli histopatolojik inceleme ve spesifik immünohistokimyasal belirteçler tanı için önemli olmasına rağmen güvenilir bir şekilde kullanılabilecek spesifik antikorlar yoktur ve biyobelirteç arayışları devam etmektedir.

Gereç ve Yöntem: Bu çalışmaya 30 adet berrak hücreli BHK ve 30 adet de papiller BHK olgusu dahil edildi. Hastalar, patolojik bir veri tabanının gözden geçirilmesiyle geriye dönük olarak tanımlandı. Patolojik veriler hastane tıbbi arşivlerinden ve patoloji raporlarından elde edildi.

Bulgular: DARS2, reelin ve enkurin proteinleri papiller BHK da berrak hücreli BHK ya kıyasla anlamlı derecede yüksek bulundu.

Sonuç: DARS2, reelin ve enkurin proteinlerinin papiller BHK ve berrak hücreli BHK ayrımı için potansiyel birer biyobelirleyiciler olabileceği sonucuna varılmıştır.

Anahtar Kelimeler: DARS, Reelin, Enkurin, papiller böbrek hücreli karsinom, berrak hücreli böbrek hücreli karsinom, biyobelirteç, immunohistokimya

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INTRODUCTION

Kidney cancer consists of adenocarcinomas that emerge in the kidney parenchyma, often called Renal Cell Carcinomas (RCCs). RCCs consist of various subtypes that have different tumor histologies, chromosomal changes, and different molecular pathways and 60-70% of RCC cases are clear-cell carcinomas.[1,2] Its microscopy consists of prominent cytoplasmic structures surrounded by thin-walled vessels, often arranged in layers, tumor cells with membranes, transparent cytoplasm, nuclei of varying sizes, and the nucleolus of which varies according to the grade of the tumor. [3]

Papillary RCC is the second most common type of RCC in the kidney and is more common in men. In microscopy, single-row or pseudostratified tumor cells lining the papillary structures containing fibrovascular cores are detected. The tumors in Type 1 Papillary Tumor cells in RCC have an ovoid nucleus, indistinct nucleoli, pale cytoplasm, and edematous cores containing histiocytes. Tumor cells are larger and their cytoplasm is abundant with eosinophilic appearance and prominent nucleoli in Type 2 papillary RCC.[4]

The differential diagnosis of a kidney tumor that shows the papillary structure and consists of cells with clear cytoplasm can be quite difficult for pathologists.[3]

Correct diagnosis is crucial for the management of patients because biological behaviors differ depending on the histological subtype. The diagnosis of the RCC subtypes can usually be achieved by careful histological and immunohistochemical (IHC) examination. However, the search for novel markers for diagnosis continues.

DARS2 is a newly identified protein contributing to high mitochondrial efficiency, and its association with tumors has been the subject of many studies conducted to date.[5,6]

Reelin, on the other hand, is a glycoprotein that is critical for neuronal positioning, migration, and synaptic activity in the brain as a novel molecule that has been suggested to suppress the invasion in tumors.[7]

Encurin is a protein that has been shown to have significant anti-cancer effects, especially in lung and colorectal cancers, nasopharyngeal cancers nasopharyngeal carcinoma, and is localized in the Ca2+ ion channel.[8]

The purpose of the study was to determine the roles of novel molecules DARS2, Reelin, and Encurin in the differential diagnosis of Papillary RCC and Clear-Cell RCC.

MATERIAL AND METHOD

The study was approved by the Fırat University Non-Interventional Health Research Ethics Committee (Date: 15.12.2022 Decision No: 2022/14-14). A total of 30 Clear-Cell RCC and 30 Papillary RCC cases were included in the study. Patients were identified retrospectively by reviewing a pathological database and the pathological data were obtained from hospital medical archives and pathology reports.

Immunohistochemistry

Immunohistochemical procedures were used as previously described by Kocaman and Artas.[9] Immunohistochemistry (IHC) was performed by using 3 µm-thick histological tissue microarray slides. The following antibodies were also used; Anti AspRS antibody (Sc-166535, Santa Cruz Biotechnology, Oregon, USA) and anti-Reelin antibody (Sc, MyBioSource, Santa Cruz Bioyechnology, Oregon, USA), and Polyclonal Antibody ENKUR (PA5-58028, ThermoFisher Waltham, Massachusetts, USA). A histoscore was calculated for the measurement of tissue levels of DARS2, Reelin, and Encurin by using indirect immunohistochemical staining.

Microscopic evaluation of staining intensity

The staining distribution was scored as 0.1, < 25%; 0.4%, 26-50%; 0.6%, 51-75%; 0.9, 76-100%, and staining intensity 0, no staining; 0.5, very little staining; 1, little staining; 2, moderate staining; 3, very strong staining. A histoscore was calculated as histoscore = distribution × intensity.[9]

Statistical Method

The data were evaluated with the Statistical Package for social sciences for Windows version 22.0 program (SPSS, Chicago, IL). The descriptive data were expressed as mean±standard error and numbers. The distribution property of the data was evaluated with the Shapiro-Wilk Test. An Independent t-test was used to compare the data that showed normal distribution. The significance level was evaluated as $p<.05$.

RESULTS

Histopathological Results

In the histopathological examination of Clear-cell RCC, a tumor with congested capillary vascular network, prominent cytoplasmic borders, clear cytoplasm, hyperchromatic nucleus, and solid cell nests was observed (**Table 1a**).

On the other hand, papillary RCC showed a tumor characterized by papillary structures with clear-pale eosinophilic cytoplasm, prominent cytoplasmic borders, lined with cells with hyperchromatic nuclei, and anastomosing fibrovascular cores (**Table 1b**).

Table 1. Histoscores of DARS2, Reelin, and Enkurin

	*Papillary RCC	**Clear Cell RCC	P*
DARS2	2.40±0.43	0.94±1.55a	.000
Reelin	1.09±0.15	0.79±0.20a	.000
Enkurin	0.15±0.06	.000a	.000

Values are given as median, min-max. a Compared to the papillary RCC group (p< .05).* Independent t test, *Papillary cell RCC: Papillary cell renal cell carcinoma, **Clear cell RCC: Clear cell renal cell carcinoma

Immunohistochemical Results

DARS2, reelin and encurin immunoreactivity: The following results were obtained as a result of the examination of the immunohistochemical staining used for immunoreactivity under light microscopy for DARS2, Reelin, and Encurin.

As seen in **Table 1**, DARS2 expression was detected in Papillary RCC and clear cell RCC groups. DARS2 expression was mostly observed in papillary RCC. When DARS2 expression was compared between groups, it was observed that it differentiated papillary RCC from clear cell RCC and the difference between them was statistically significant (**Figure 1A-1D, 2a-2d**) (**Table 1**) (p< .05).

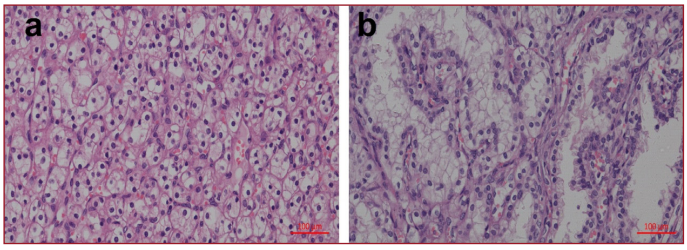

Figure 1. Hematoxylin-eosin image of *papillary cell RCC and **clear cell RCC lesion areas.

*Papillary cell RCC: Papillary cell renal cell carcinoma
**Clear cell RCC: Clear cell renal cell carcinoma

The expression of reelin and encurin proteins was highest in papillary RCC. Reelin expression was found to be significantly lower in clear cell RCC compared to papillary RCC (**Figure 1B-1E, 2b-2e**) (p< .05), Encurin expression was not observed in clear cell RCC (**Figure 1C-1F, 2c-2f**).) (p< .05). The difference in expression between the groups was found to be statistically significant (**Table 1**) (p< .05).

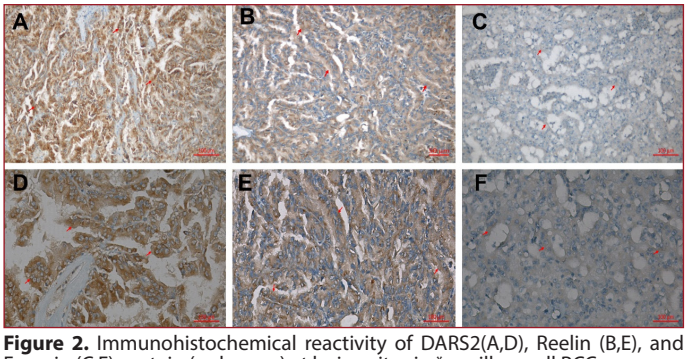

Figure 2. Immunohistochemical reactivity of DARS2(A,D), Reelin (B,E), and Encurin (C,F) protein (red arrow) at lesion sites in *papillary cell RCC.

*Papillary cell RCC: Papillary cell renal cell carcinoma

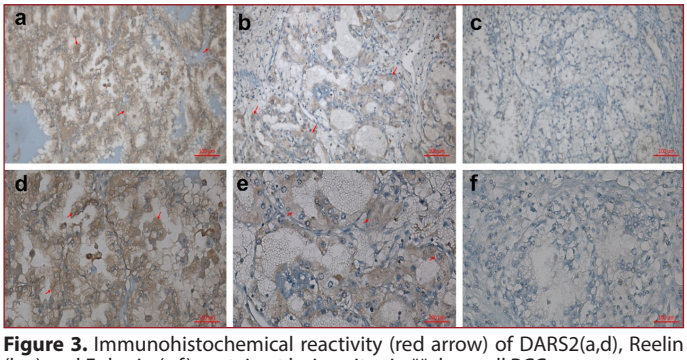

Figure 3. Immunohistochemical reactivity (red arrow) of DARS2(a,d), Reelin (b,e) and Enkurin (c,f) protein at lesion sites in **clear cell RCC.

**Clear cell RCC : Clear cell renal cell carcinoma

DISCUSSION

The typing of a kidney tumor that consists of cells with papillary structure and clear cytoplasm can be challenging for pathologists. As clear-cell RCC sometimes shows the papillary structure, papillary RCC may also be clear-cell in some cases. Accurate identification of these different entities is important in prognostic and therapeutic terms. Although histomorphological characteristics in routine Hematoxylin-Eosin Staining are the gold standard method in reaching the correct diagnosis in many cases, immunohistochemical studies are life-saving as an auxiliary diagnostic method for cases with difficult differential diagnosis.[3] Although many IHC markers have been investigated for this purpose to date, unfortunately, a specific biomarker that can be used for problematic cases has not yet been found.

DARS2, Reelin, and Encurin proteins were investigated as IHC in papillary and clear-cell RCC in the present study. Although DARS2 was reactive for both tumors, it was found to be significantly increased in papillary RCC.

Normally, mitochondrial dysfunction causes an increase in the production of reactive oxygen derivatives, which often leads to the accumulation of oxidized proteins in the cell and the regulation of antioxidant responses. DARS2 is a mitochondrial protein, and it is already known that pathologies in the DARS2 gene cause direct disruption of mitochondrial protein synthesis and tissue-specific activation of cellular stress responses.[10] In a previous study, it was determined that mitochondrial dysfunction causes reactive oxygen release that mediates epithelial-mesenchymal transition through intracellular signal transduction and cell invasion in lung cancer and attention was drawn to the relationship of DARS2 with tumorigenesis.[11]

In our previous study, it was shown that there is a relationship between DARS2 and adenocarcinoma of the lung and malignant mesothelioma, and it was suggested that it can be used as a biomarker for the differential diagnosis of these 2 tumors.[12]

The findings of this study were also very important in terms of demonstrating that DARS2 is a marker that can be used in the differential diagnosis of papillary and clear cell RCCs because mitochondrial mechanisms that affect tumorigenesis were studied recently, and some specific nuclear mitochondrial genes are considered to be potential targets for the development of next-generation cancer therapeutics.[6]

Reelin protein is associated with various brain disorders such as Alzheimer's Disease, schizophrenia, and depression, and its expression was also described in non-neuronal tissues such as the liver, breast, and kidney in more recent studies.[13] Reelin gene expression is regulated by various genetic and epigenetic mechanisms. In a recent study, it was stated that an increase in reelin prevents the development of colon cancer, and its decrease might trigger the formation of colon carcinoma, and it was suggested as a biomarker in the prediction of prognosis.[14]

In the present study, it was found that reelin was more expressed in papillary RCC, but the expression decreased in clear cell carcinoma, which is a more aggressive tumor, compared to papillary RCC, and the difference between them was statistically significant. Decreased expression of reelin in clear cell RCC, which is quite aggressive, is a finding that was expected, and it was in line with the literature data. In a different study, the antitumor effect of reelin was emphasized by showing that blockade of reelin expression increases tumor aggressiveness in breast cancer, in line with the findings of the present study.[15]

Encurin, on the other hand, is a Ca2 channel protein and was also found to induce an anti-tumor effect in the proliferation and metastasis of cancer cells by binding to β-catenin and suppressing the nucleocytoplasmic transport of β-catenin.[16] It was also shown that encurin, which is also effective as a tumor suppressor protein, inhibits proliferation, migration, and invasion of non-small cell lung cancer cells, and the absence of encurin accelerates tumor progression.[17] It was reported in previous studies that encurin plays tumor suppressor roles in lung adenocarcinoma cells through PI3K/Akt signaling pathways, and it was argued that encurin-targeted therapies would be promising for patients.[18] It is already known that the members of the Protein Kinase C (PKC) family contribute to intracellular signaling in cancer, and one of its subforms, Protein Kinase Cε (PKCε), was accepted as an oncogene. The overexpression of PKCε plays critical roles in different processes leading to cancer development, including RCC. It was shown that increased PKCε expression correlates with tumor grade in RCC and PKCε regulates cell proliferation. Its effects on the invasion ability, migration, and chemo-resistance of tumor cells in Clear-cell RCC were investigated and it was emphasized that PKCε is important for the survival of tumor cells in Clear-cell RCC.[19] In the present study, it was found that encurin was secreted very little in papillary RCC, but not at all in Clear-cell RCC, which is a very aggressive tumor. As the aggression increases, the decreased secretion of encurin may be a finding that can be used in the differential diagnosis of papillary and Clear-cell RCCs, as well as a guide for encurin-targeted treatment procedures in RCCs.

Of course, the study also had limitations, as in many other studies. The most important limitation was that it had a retrospective design, and therefore, Western Blot Analyzes of the proteins examined were not performed. Also, not including grades of tumoral tissues in the study was another limitation. For this purpose, studies to be conducted in larger series will reveal the relationship of DARS2, reelin, and encurin proteins with papillary and clear cell BCCs.

CONCLUSION

It was determined in the present study that DARS2, reelin, and encurin proteins may be biomarkers in the differential diagnosis of papillary and Clear-cell RCCs. Decreased levels of reelin and encurin in tumoral tissues in RCC were determined to increase aggressiveness, and they were shown to be candidate molecules for the development of new cancer therapeutics specific to these tumors.

ETHICAL DECLARATIONS

Ethics Committee Approval: The permission was received with the date 01.12.2022 and number 2022/14-14 from Fırat University Non-Interventional Health Research Ethics Committee.

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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