EKSPRESIJA *NCAM* I *FGFR1* MOLEKULA I NJIHOV UTICAJ NA BIOLOŠKO PONAŠANJE KARCINOMA BUBREŽNIH ĆELIJA

ORIGINALNI RAD

ORIGINAL ARTICLE

POSSIBLE IMPACT OF NCAM AND FGFR1 MOLECULE EXPRESSION PATTERNS ON THE BIOLOGICAL BEHAVIOR OF RENAL CELL CARCINOMA

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SAŽETAK

Uvod: Poslednjih decenija raste stopa javljanja tumora bubrežnih ćelija (engl. *renal cell tumors - RCT*) i njima uzrokovanih smrtnih ishoda. Iako karcinomi bubrežnih ćelija (*engl. renal cell carcinoma – RCC*) predstavljaju samo 2% svih karcinoma, ovi tumori spadaju u prvih deset uzročnika smrti među karcinomima u Evropi.
Cilj: Kako je poznato da neuralni ćelijski adhezioni molekul (engl. *neural cell adhesion molecule, NCAM*) i receptor 1 za fibroblastni faktor rasta (engl. *fibroblast growth factor receptor 1, FGFR1*) stupaju u interakcije na površini ćelijske membrane, kao i da se mogu eksprimirati i na drugim ćelijskim lokalizacijama, odlučili smo da ispitamo potencijalni uticaj različitih obrazaca njihove koekspresije na kliničko-patološke karakteristike tumora bubrega.
Materijal i metode: Analizirano je 100 tumora bubrega, dijagnostikovanih na Institutu za Patologiju Medicinskog fakulteta Univerziteta u Beogradu. Imunohistohemijska analiza urađena je na pločicama tkivnog mikroniza, korišćenjem *NCAM* (1:50, klon123C3.D5) i *FGFR1* (1:100, klon M19B2) antitela. Kliničke i patohistološke karakteristike tumora bubrega ispitane su u odnosu na prisustvo i lokalizaciju koekspresije *NCAM i FGFR1* molekula.
Rezultati: Koekspresija *NCAM i FGFR1* molekula u tumorima bubrega uočena je u citoplazmi i na membrani, ali ovi obrasci ne zavise od patohistološkog tipa tumora. Svaki tumor u čijem jedru je uočena *FGFR1* imunopozitivnost pokazivao je i membransku pozitivnost na oba ispitivana molekula. Primećeno je da sa povećanjem T stadijuma raste učestalost koekspresije *NCAM i FGFR1* molekula, ali nalaz nije bio statistički značajan.
Zaključak: Membranska koekspresija nije uočena ni kod jednog benignog tumora, uprkos prisustvu citoplazmatske koekspresije. Postoji mogućnost i da pojava *FGFR* molekula u jedru indukuje pojavu membranske koekspresije.

Ključne reči: *RCT, RCC, NCAM, FGFR1*, tumori bubrega

ABSTRACT

Introduction: The incidence of renal cell tumors (RCT) and the deaths caused by them has been increasing in recent decades. Although renal cell carcinomas (RCCs) represent only 2% of all cancers, these tumors are among the top ten causes of death in Europe, when cancers are concerned.
Aim: As it is known that the neural cell adhesion molecule (NCAM) and fibroblast growth factor receptor 1 (FGFR1) interact on the surface of the cell membrane and can also be expressed in other cellular localizations, we decided to examine the potential influence of different patterns of their co-expression on the clinical and pathological characteristics of renal tumors.
Material and methods: A total of 100 renal tumors, diagnosed at the Institute of Pathology, Faculty of Medicine, University of Belgrade, were analyzed. Immunohistochemical analysis was performed on tissue microarray slides, using NCAM (1:50, clone123C3.D5) and FGFR1 (1:100, clone M19B2) antibodies. Clinical and pathohistological characteristics of renal tumors were examined in relation to the presence and localization of the co-expression of NCAM and FGFR1 molecules.
Results: Co-expression of NCAM and FGFR1 molecules in renal tumors was observed in the cytoplasm and on the membrane, however, these patterns did not depend on the pathohistological type of tumor. Each tumor in which FGFR1 immunopositivity was observed in the nucleus also showed membranous positivity for both tested molecules. It was observed that the frequency of co-expression of NCAM and FGFR1 molecules increased with increasing T stage, but the finding was not statistically significant.
Conclusion: Membranous co-expression was not observed in any benign tumor, despite the presence of cytoplasmic co-expression. There is also a possibility that the presence of FGFR in the nucleus induces the occurrence of membranous co-expression.

Keywords: RCT, RCC, NCAM, FGFR1, renal tumors

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UVOD

Tokom poslednje tri decenije, incidencija tumora bubrega je u stalnom porastu u Evropi, SAD-u i Australiji [1,2]. U adultnoj populaciji, karcinomi bubrega čine 2% svih karcinoma [3], a najčešći je karcinom bubrežnih ćelija (engl. *renal cell carcinoma, RCC*), koji ima najveći mortalitet među karcinomima urogenitalnog sistema [4]. Izdvaja se nekoliko patohistoloskih podtipova, među kojima postoje razlike u morfologiji, genetici, poreklu i biološkom ponašanju. Ubedljivo je najčešći svetloćelijski (80 – 90%) karcinom, za kojim slede papilarni, hromofobni i karcinom sabirnih kanalića [5]. Iako su među tumorima bubrežnih ćelija najčešći karcinomi, postoje i benigni tumori poznati kao onkocitomi [6]. Godišnje se širom sveta dijagnostikuje oko 270.000 novih slučajeva *RCC*-a godišnje, a oko 116.000 pacijenata umire [1,2]. S obzirom na činjenicu da ovi tumori retko pokazuju rane znake bolesti (što rezultira visokom proporcijom pacijenata sa metastazama), kao i da se karakterišu raznolikim kliničkim manifestacijama, visokom rezistencijom na radioterapiju i hemioterapiju [7], sprovedena su mnoga kliničko-patološka ispitivanja u cilju otkrivanja potencijalnih biomarkera, kako bi se omogućila rana dijagnostika i imunomodulacija u cilju inhibicije tumorskog rasta.

Neuralni ćelijski adhezioni molekul (engl. *neural cell adhesion molecule, NCAM*) je transmembranski protein, eksprimiran u mnogim tkivima tokom organogeneze. Značajnu ulogu tokom embrionalnog razvoja ostvaruje ne samo u nervnom, mišićnom, neuroektodermalnom i neuroendokrinom tkivu, već i u organima drugačijeg porekla, uključujući i bubrege, igrajući važnu ulogu u procesu mezenhimno-epitelne transformacije (MET), migracije i proliferacije [8]. U adultnom bubregu, *NCAM* je prisutan samo u retkim intersticijumskim ćelijama [9], kao i u bubrežnim neoplazmama [10].

Receptor za fibroblastni faktor rasta (engl. *fibroblast growth factor receptor, FGFR*) pripada familiji tirozin-kinaznih receptora, značajnih za ćelijsku proliferaciju i migraciju, diferencijaciju, apoptozu, epitelno-mezenhimnu transformaciju (EMT) i kancerogenezu [11–14]. *FGFR* receptori mogu biti aktivirani i nekim transmembranskim molekulima, uključujući *NCAM* [15]. Interakcije ova dva molekula opisane su u nervnom [16] i ne-nervnom tkivu [17], ali i u tumorima [18].

Osim podataka da svaki od ova dva molekula učestvuje u procesima migracije, proliferacije i procesima epitelno-mezenhimne transformacije (EMT), koji dovode do transformacije normalne epitelne ćelije u neoplastičnu ćeliju mezenhimalnih karakteristika, postoje podaci i o njihovoj interakciji, kao faktoru koji pospešuje invazivni potencijal pojedinih karcinoma [19]. Interakcija *NCAM* i *FGFR* molekula, njihova ekspresija u

INTRODUCTION

Over the past three decades, the incidence of renal tumors has been steadily increasing in Europe, USA, and Australia [1,2]. In the adult population, kidney cancers account for 2% of all cancers [3], with the most common one being renal cell carcinoma (RCC), which has the highest mortality among urogenital system cancers [4]. There are several pathohistological subtypes, among which there are differences in morphology, genetics, origin, and biological behavior. Clear cell carcinoma is by far the most common (80% – 90%) type, followed by papillary, chromophobe, and collecting duct carcinoma [5]. Although the most common renal cell tumors are carcinomas, there are also benign tumors known as oncocytomas [6]. About 270,000 new cases of RCC are diagnosed annually worldwide, and about 116,000 patients die [1,2]. Given the fact that these tumors rarely show early signs of disease (resulting in a high proportion of patients with metastases), as well as the fact that they are characterized by diverse clinical manifestations and increased resistance to radiotherapy and chemotherapy [7], many clinical and pathological examinations have been conducted for the purpose of discovering potential biomarkers and enabling early diagnosis and immunomodulation with the aim of inhibiting tumor growth.

Neural cell adhesion molecule (NCAM) is a transmembrane protein expressed in many tissues during organogenesis. It plays a significant role during embryonic development, not only in nerve, muscle, neuroectodermal and neuroendocrine tissue, but also in organs of different origin, including kidneys, with an important role in the process of mesenchymal-epithelial transformation (MET), migration and proliferation [8]. In the adult kidney, NCAM is present only in rare interstitial cells [9], as well as in renal neoplasms [10].

The fibroblast growth factor receptor (FGFR) belongs to the family of tyrosine-kinase receptors, important for cell proliferation and migration, differentiation, apoptosis, epithelial-mesenchymal transition (EMT) and carcinogenesis [11–14]. FGFR receptors can also be activated by some transmembrane molecules, including NCAM [15]. The interactions between these two molecules have been described in nervous [16] and non-nervous tissue [17], but also in tumors [18].

Apart from data supporting the fact that each of these two molecules participates in the processes of migration, proliferation, as well as processes of epithelial-mesenchymal transition (EMT), leading to the transformation of a normal epithelial cell into a neoplastic cell with mesenchymal characteristics, there are also data on their interaction as a factor enhancing the invasive potential of certain carcinoma [19]. The

proliferišućim tumorskim ćelijama i metastazama različitih tumora, ali i opisana ekspresija u bubrežnim neoplazmama, čini ove molekule, kao površinske markere, pogodnim za uspostavljanje dijagnoze, ali i potencijalnom metom za primenu novih terapijskih modaliteta [20 – 28].

Kako je poznato da *NCAM* i *FGFR* stupaju u interakcije na površini ćelijske membrane, odlučili smo da ispitamo potencijalni uticaj različitih obrazaca njihove koekspresije na kliničko-patološke karakteristike tumora bubrežnih ćelija.

MATERIJALI I METODE

Uzorci tkiva za analizu i tkivni mikroniz

Iz parafinskih kalupa tkiva tumora bubrega, dijagnostikovanih u periodu 2010 – 2013. godine na Institutu za patologiju Medicinskog fakulteta Univerziteta u Beogradu, uzeti su cilindri tkiva za pravljenje tkivnog mikroniza. Uzorkovanje je rađeno korišćenjem šuplje medicinske igle prečnika 0,6 mm, iz parafinskih kalupa tumora bubrežnih ćelija. Iz svakog kalupa su uzeta tri tkivna cilindra koja su zatim ubačena u parafinski blok i precizno raspoređena u obliku niza. Pomoću mikrotoma, parafanski kalupi tkivnog mikroniza su sečeni na isečke debljine 5 µm i postavljani na mikroskopske pločice, koje su dalje korišćene za imunohistohemijsku analizu.

Uzorci tumorskog tkiva su dobijeni iz 100 tumora bubrega, među kojima je bilo 69 svetloćelijskih *RCC*-a, 12 papilarnih *RCC*-a, 7 hromofobnih *RCC*-a, 5 multilokularnih cističnih *RCC*-a, dva karcinoma Belinijevih sabirnih kanalića, kao i 5 onkocitoma.

Imunohistohemija

Imunohistohemija je urađena na pločicama tkivnih mikronizova. Nakon deparafinizacije u ksilolu i hidratacije, pločice su ubačene u citratni pufer (pH 6,0) i izložene mikrotalasima u trajanju od 20 min na 400 W. Blokada peroksidazne aktivnosti je izvršena sa 1% govođim serumskim albuminom (engl. *bovine serum albumin – BSA*). Nakon ekstrakcije antigena, urađena je inkubacija sa primarnim antitelima *NCAM* (1:50, klon 123C3. D5, *LabVision, USA*) i *FGFR1* (1:100, klon M19B2, *Abcam, USA*) u trajanju od jednog sata. *EnVision™* (*DAKO*, Danska) je korišćen za vizuelizaciju antigen-antitelo reakcije sa 3,3'-diaminobenzidinom (DAB) i sledstvenim kontrastiranjem sa hemalaunom (*Merc, USA*). Negativne kontrole su dobijene izostavljanjem primarnog antitela. Pločice su pregledane upotrebom *BX53* svetlosnog mikroskopa sa *DP12CCD* kamerom (*Olympus*, Nemačka).

interaction between NCAM and FGFR molecules, their expression in proliferating tumor cells and metastases of various tumors, but also the described expression in renal neoplasms, makes these molecules, as surface markers, suitable for establishing a diagnosis, but also makes them a potential target for the application of new therapeutic modalities [20 – 28].

As NCAM and FGFR are known to interact on the surface of the cell membrane, we decided to investigate the potential impact of different patterns of their co-expression on the clinicopathological characteristics of renal cell tumors.

MATERIALS AND METHODS

Tissue samples for analysis and tissue microarray

Sample cylinders of tissue were taken from paraffin molds of renal tumor tissue, diagnosed in the period 2010 – 2013 at the Institute of Pathology of the Faculty of Medicine, University of Belgrade, for making a tissue microarray. Sampling was performed from paraffin molds of renal cell tumors with a hollow needle (0.6 mm in diameter). Three tissue cylinders were taken from each mold, which were then embedded in a paraffin block and precisely arranged in an array. Using a microtome, tissue microarray paraffin molds were cut into 5 µm thick sections and mounted on microscope slides, which were further used for immunohistochemical analysis.

Tumor tissue samples were obtained from 100 renal tumors, among which there were 69 clear cell RCCs, 12 papillary RCCs, 7 chromophobe RCCs, 5 multilocular cystic RCCs, two Bellini duct carcinomas, and 5 oncocytomas.

Immunohistochemistry

Immunohistochemistry was performed on tissue microarray slides. After deparaffinization in xylene and hydration, the slides were placed in a citrate buffer (pH 6.0) and exposed to microwaves for 20 min, at 400 W. Peroxidase activity was blocked with 1% bovine serum albumin (BSA). After antigen extraction, incubation with primary NCAM antibodies (1:50, clone 123C3.D5, LabVision, USA) and FGFR1 (1:100, clone M19B2, Abcam, USA) was performed for one hour. EnVisionTM (DAKO, Denmark) was used to visualize the antigen-antibody reaction with 3,3'-diaminobenzidine (DAB) and subsequent contrast with hemalaun (Mertz, USA). Negative controls were obtained by excluding the primary antibody. Plates were examined using a BX53 light microscope with a DP12CCD camera (Olympus, Germany).

Statistička analiza

Statistička analiza je izvršena upotrebom *IBM SPSS* softvera, verzije 20.0. Korišćeni su χ2 test, Fišerov test, Studentov *t* test, Men-Vitnijev U test, Kruskal-Valisov test i *ANOVA* test, a vrednost $p < 0,05$ je smatrana statistički značajnom. Demografske, kliničke i patohistološke karakteristike tumora bubrega (pol pacijenta, veličina tumora, tip tumora, nuklearni gradus i TNM stadijum bolesti) ispitane su u odnosu na prisustvo i lokalizaciju koekspresije *NCAM* i *FGFR1* molekula.

REZULTATI

Analizirajući 100 tumora, od čega je 68 pripadalo pacijentima muškog, a 32 ženskog pola, istovremenu ekspresiju *NCAM* i *FGFR1* molekula smo zabeležili u 77 tumora (87,8%).

NCAM molekul je uočen na membrani i u citosolu, dok je *FGFR1* uz ove lokalizacije obuhvatio i jedarnu distribuciju.

Kod većine patohistoloških tipova, učestalost koekspresije je bila u rasponu 80% – 100%, dok je od dva karcinoma Belinijevih sabirnih kanalića, samo jedan pokazivao istovremenu ekspresiju *NCAM* i *FGFR1* molekula (Tabela 1).

Primetili smo da je porast javljanja koekspresije pratila porast nuklearnog gradusa, iako nije bilo statističke značajnosti, kao i da su svi tumori sa najvišim nuklearnim gradusom istovremeno eksprimirali ove molekule (Tabela 1).

Statistical analysis

Statistical analysis was performed using IBM SPSS software, version 20.0. The χ2 test, Fisher's test, Student's t test, Mann-Whitney U test, Kruskal-Wallis test, and the ANOVA test were used, and a value of $p < 0.05$ was considered statistically significant. Demographic, clinical and pathohistological characteristics of renal tumors (patient gender, tumor size, tumor type, nuclear grade, and TNM stage of the disease) were examined in relation to the presence and localization of the co-expression of NCAM and FGFR1.

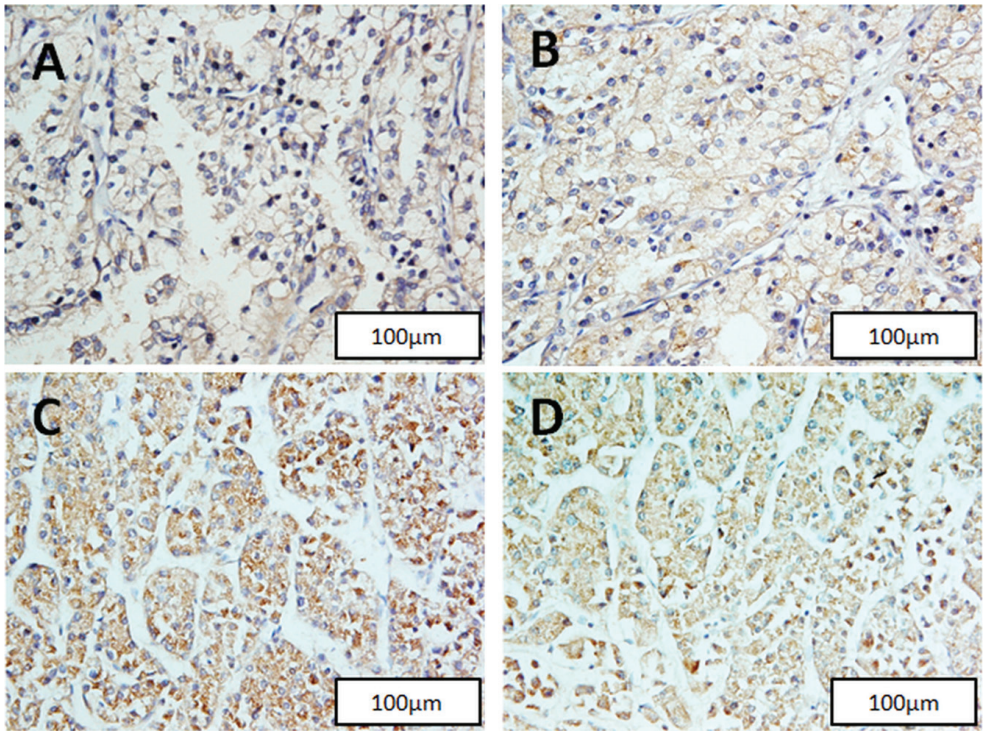
RESULTS

The analysis of 100 tumors, of which 68 were found in male patients and 32 in female patients, we recorded the simultaneous expression of NCAM and FGFR1 in 77 tumors (87.8%).

The NCAM molecule was observed on the membrane and in the cytosol, while FGFR1 included nuclear distribution in addition to these localizations.

In most pathohistological types, the frequency of co-expression was in the 80% – 100% range, while of the two Bellini duct carcinomas, only one showed simultaneous expression of NCAM and FGFR1 (Table 1).

We observed that the increase in the occurrence of co-expression followed the increase in nuclear grade, although without statistical significance, and that all tumors with the highest nuclear grade simultaneously expressed these molecules (Table 1).



Slika 1. Prikaz membranske koekspresije *NCAM* (A) i *FGFR1* (B) molekula u svetloćelijskom RCC-u i citoplazmatske imunoreaktivnosti oba molekula u onkocitomu (*C – NCAM, D – FGFR1*)

Figure 1. Representation of membrane co-expression of NCAM (A) and FGFR1 (B) molecules in clear cell RCC and of cytoplasmic immunoreactivity of both molecules in oncocytoma (C – NCAM, D – FGFR1)

Tabela 1. Patohistološke karakteristike tumora bubrega u odnosu na prisustvo FGFR1-NCAM koekspresije

Table 1. Pathohistological characteristics of renal tumors in relation to the presence of FGFR1-NCAM co-expression

Patohistološke karakteristike / *Pathohistological characteristics*		Koekspresija FGFR1-NCAM / *FGFR1-NCAM co-expression* n (%)		p-vrednost/ *p-value*
		Odsutna / *Absent*	Prisutna / *Present*	
Tip tumora / *Type of tumor*	Svetloćelijski RCC / *Clear cell RCC*	9 (15%)	51 (85%)	0.383
	Papilarni RCC, niski gradus / *Papillary RCC, low grade*	0 (0.0%)	2 (100.0%)	
	Papilarni RCC, visoki gradus / *Papillary RCC, high grade*	0 (0%)	9 (100%)	
	Multilokularni cistični RCC / *Multilocular cystic RCC*	1 (20%)	4 (80%)	
	Hromofobni RCC / *Chromophobe RCC*	0 (0.0%)	6 (100.0%)	
	Karcinom Belinijevih sabirnih kanalića / *Bellini duct carcinoma*	1 (50%)	1 (50%)	
	Onkocitom / *Oncocytoma*	0 (0.0%)	5 (100.0%)	
Nuklearni gradus / *Nuclear grade* (NG)	NG I	1 (14.3%)	6 (85.7%)	0.821
	NG II	6 (15.4%)	33 (84.6%)	
	NG III	4 (12.9%)	27 (87.1%)	
	NG IV	0 (0%)	5 (100%)	
T stadijum / *T stage*	T1	7 (22.6%)	24 (77.4%)	0.121
	T2	2 (22.2%)	7 (77.8%)	
	T3	1 (3.1%)	31 (96.9%)	
	T4	0 (0.0%)	1 (100%)	
N stadijum / *N stage*	N0	0 (0.0%)	8 (100%)	1.000
	N1	0 (0.0%)	2 (100.0%)	
M stadijum / *M stage*	M0	0 (0.0%)	1 (100.0%)	1.000
	M1	0 (0.0%)	2 (100.0%)	

n – broj slučajeva; N0 – bez regionalnih metastaza; N1 – sa regionalnim metastazama; M0 – bez sistemskih metastaza; M1 – sa sistemskim metastazama

n – number of cases; N0 – without regional metastases; N1 – with regional metastases; M0 – without systemic metastases; M1 – with systemic metastases

Sa povećanjem T stadijuma uočen je porast učestalosti istovremene ekspresije *NCAM* i *FGFR1* molekula u tumorima bubrega. Među tumorima viših stadijuma (T3 i T4), samo jedan tumor nije pokazao koekspresiju, dok koekspresije nije bilo u 22,9% tumora u stadijumima T1 i T2. Međutim, nije bilo statistički značajne razlike (Tabela 1).

Budući da nam podaci o nodalnim i sistemskim metastazama za veći broj uzoraka nisu bili dostupni, nismo bili u prilici da ispitamo odnos njihovog postojanja i prisustva koekspresije. Ipak, uočili smo da su među tumorima o kojima smo dobili ove podatke, svi tumori sa metastazama, kao i svi tumori bez metastaza, pokazali koekspresiju (Tabela 1).

Prisustvo membranske ekspresije i *NCAM* i *FGFR1* molekula zapazili smo kod svega 59% tumora, pri čemu je ona bila vrlo varijabilna u odnosu na patohistološki tip tumora. Ni kod jednog tumora među karcinomi-

With an increasing T stage, an increase in the frequency of simultaneous expression of NCAM and FGFR1 in renal tumors was observed. Among high stage tumors (T3 and T4), only one tumor showed no co-expression, while co-expression was absent in 22.9% of T1 and T2 stage tumors. However, there was no statistically significant difference (Table 1).

Since data on nodal and systemic metastases for a larger number of samples were not available to us, we were unable to examine the relationship between their occurrence and the presence of co-expression. However, we observed that among the tumors for which we had obtained this data, all tumors with metastases, as well as all tumors without metastases, showed co-expression (Table 1).

We observed the presence of membranous expression of both NCAM and FGFR1 in only 59% of tumors, with great variations depending on the pathohistolog-

Tabela 2. Patohistološke karakteristike tumora bubrega u odnosu na lokalizaciju FGFR1-NCAM koekspresije

Table 2. Pathohistological characteristics of renal tumors in relation to the presence of FGFR1-NCAM co-expression

Patohistološke karakteristike / *Pathohistological characteristics*		Membranska lokalizacija koekspresije FGFR1-NCAM / *Membranous localization of FGFR1-NCAM co-expression* n (%)		p-vrednost/ *p-value*
		Odsutna / *Absent*	Prisutna / *Present*	
Tip tumora / *Type of tumor*	Svetloćelijski RCC / *Clear cell RCC*	24 (40%)	36 (60%)	0.372
	Papilarni RCC, niski gradus / *Papillary RCC, low grade*	1 (50.0%)	1 (50.0%)	
	Papilarni RCC, visoki gradus / *Papillary RCC, high grade*	3 (33.3%)	6 (66.7%)	
	Multilokularni cistični RCC / *Multilocular cystic RCC*	1 (20%)	4 (100%)	
	Hromofobni RCC / *Chromophobe RCC*	2 (28.6%)	5 (71.4%)	
	Karcinom Belinijevih sabirnih kanalića / *Bellini duct carcinoma*	2 (100%)	0 (0%)	
	Onkocitom / *Oncocytoma*	3(75%)	1 (25%)	
Nuklearni gradus / *Nuclear grade* (NG)	NG I	2 (28.6%)	5 (71.4%)	0.854
	NG II	14 (35.9%)	25 (64.1%)	
	NG III	14 (43.8%)	18 (56.3%)	
	NG IV	2 (40%)	3 (60%)	
T stadijum / *T stage*	T1	15 (48.4%)	16 (51.6%)	0.638
	T2	4 (44.4%)	5 (56.6%)	
	T3	12 (36.4%)	21 (63.6%)	
	T4	0 (0%)	1 (100%)	
N stadijum / *N stage*	N0	2 (22.2%)	7 (77.8%)	1.000
	N1	0 (0%)	2 (100%)	
M stadijum / *M stage*	M0	0 (0%)	1 (100%)	1.000
	M1	0 (0%)	2 (100%)	

n – broj slučajeva; N0 – bez regionalnih metastaza; N1 – sa regionalnim metastazama; M0 – bez sistemskih metastaza; M1 – sa sistemskim metastazama

n – number of cases; N0 – without regional metastases; N1 – with regional metastases; M0 – without systemic metastases; M1 – with systemic metastases

ma sabirnih kanalića nismo uočili membransku koekspresiju, dok je među onkocitomima ona bila prisutna samo kod jednog pacijenta, a kod svih ostalih tipova je koekspresija bila prisutna sa učestalošću većom od 50%, kao što je prikazano u Tabeli 2. Na Slici 1 prikazani su različiti obrasci *NCAM* i *FGFR1* ekspresije.

Nije uočena povezanost nuklearnog gradusa i membranske koekspresije, ali je primećeno da je porast T stadijuma pratilo i povećanje procenta tumora koji su na membranama imali oba molekula, iako nije bilo statistički značajne razlike (Tabela 2).

Ispitivanjem povezanosti veličine tumora i lokalizacije *NCAM* i *FGFR1* koekspresije, ustanovljeno je da su tumori bubrega bez membranske koekspresije bili prosečno nešto manje veličine (6,3 ± 2,7) od onih koji su imali membransku pozitivnost (6,9 ± 4,3), $p = 0,390$.

ical type of the tumor. We did not observe membranous co-expression in any tumor among the collecting duct carcinomas, while among oncocytomas it was present in only one patient, whereas in all other types of tumors, co-expression was present with a frequency greater than 50%, as shown in Table 2. Figure 1 shows different patterns of NCAM and FGFR1 expression.

No association between nuclear grade and membranous co-expression was observed, but it was noted that the increase in T stage was followed by an increase in the percentage of tumors that had both molecules on the membranes, although there was no statistically significant difference (Table 2).

The examination of the relationship between tumor size and localization of NCAM and FGFR1 co-expression showed that renal tumors without membra-

Analizom lokalizacije ekspresije svakog od ova dva molekula, uvideli smo da imaju veoma sličnu distribuciju. Tumori sa membranskom ekspresijom *NCAM* molekula su u 83% slučajeva na membranama eksprimirali i *FGFR1*, dok je ekspresiju *FGFR1* molekula na membrani pratila i membranska ekspresija *NCAM* molekula u 86% slučajeva. Zanimljivo je da su svi tumori u čijem smo jedru našli *FGFR1* molekule imali i *FGFR1* i *NCAM* molekule na površini membrane.

DISKUSIJA

Karcinogeneza je višestepeni proces koji često kulminira invazijom tumorskih ćelija u okolno tkivo i u krvne sudove, na taj način doprinoseći njihovoj diseminaciji i u udaljena tkiva. Otkrivanje molekularnih interakcija koje omogućavaju inicijaciju i progresiju ovog procesa u mnogome bi olakšalo definisanje ključnih karika, koje bi mogle biti meta ciljnog terapijskog delovanja.

Džimbo i saradnici su otkrili da *NCAM* u različitim tumorskim ćelijama indukuje stvaranje proteina koji onemogućava pripajanje tumorskih ćelija za matriks i bazalnu membranu [29]. Ovi autori su jednom delu eksperimentalnih životinja preneli tumorske ćelije koje su eksprimirale taj protein, dok su drugom delu preneli ćelije koje nisu posedovale ovaj protein. Broj životinja kod kojih se tumor razvio nakon prenošenja je kod prve grupe bio značajno manji od broja životinja u drugoj grupi. Ovi rezultati ukazuju na to da *NCAM*, posredstvom drugog proteina za čiju je ekspresiju odgovoran, negativno utiče na procese ključne za širenje malignog procesa.

S druge strane, postoje podaci koji ukazuju na to da agresivnost neuroblastoma i neuroendokrinih tumora raste s javljanjem *NCAM* proteina [30,31]. Ovakvi, naizgled suprotstavljeni rezultati, navode na ideju da za procenu biološkog ponašanja tumora možda nije korisno ispitivanje nezavisne ekspresije ovog molekula, već da su značajni i drugi molekuli sa kojima on stupa u različite interakcije koje dovode do stimulacije ili inhibicije različitih procesa. Tako je uočeno da se kolokalizacija *NCAM* molekula i aldehid dehidrogenaze 1 (ALDH1) u blastemskoj komponenti Vilmsovog tumora javlja u 33% slučajeva, a utvrđeno je da ovakav imunomorfološki profil značajno utiče na pojavu metastaza, recidiva bolesti i smrti pacijenata, kao i da determiniše odgovor na *ifosfamide-carboplatin-etoposide* (*ICE*) protokol hemoterapije [22]. U svojoj studiji, Jang i Lu su pokazali da koekspresija *CCND1* i *FGFR1* molekula postoji kod karcinoma pluća, kao i da *FGFR1* promoviše EMT [21]. Ispitivanjem ćelija pleuropulmonalnog blastoma, Šukrun i Golan su primetili da tretiranjem anti-*NCAM* imunokonjugatom dolazi do supresije rasta tumorskih ćelija u ovom tumoru [23]. Egbivi i Kokl su u

nous co-expression were, on average, slightly smaller in size (6.3 ± 2.7) than those with membranous positivity (6.9 ± 4.3), $p = 0.390$.

By analyzing the localization of the expression of each of these two molecules, we saw that they had very similar distribution. Tumors with membranous expression of NCAM also expressed FGFR1 on the membranes in 83% of cases, while the expression of FGFR1 on the membrane was accompanied by membranous expression of NCAM in 86% of the cases. It is interesting that all tumors in whose nucleus we found FGFR1 molecules, had both FGFR1 and NCAM molecules on the membrane surface.

DISCUSSION

Carcinogenesis is a multistage process that often culminates in the invasion of tumor cells into the surrounding tissue and blood vessels, thus contributing to their dissemination to distant tissues. The discovery of molecular interactions that enable the initiation and progression of this process would greatly facilitate the defining of key links, which could be the focus of targeted treatment.

Jimbo et al. found that NCAM in various tumor cells induces the production of a protein that prevents the attachment of tumor cells to the matrix and the basement membrane [29]. These authors transferred tumor cells expressing this protein to one group of experimental animals, while they transferred cells that did not possess this protein to the other group of experimental animals. The number of animals developing tumors after transfer was significantly lower in the first group than in the second group. These results indicate that NCAM, via another protein whose expression it is responsible for, has a negative effect on the processes crucial for the dissemination of the malignant process.

On the other hand, there are data indicating that the aggressiveness of neuroblastoma and neuroendocrine tumors increases with the presence of the NCAM protein [30,31]. These seemingly contradictory results indicate that for the assessment of the biological behavior of tumors it may not be useful to examine the independent expression of this molecule, but that other molecules, with which NCAM enters into various interactions that lead to the stimulation or inhibition of various processes, are also important. Thus, it was observed that the colocalization of the NCAM molecule and aldehyde dehydrogenase 1 (ALDH1) in the blastema component of Wilms' tumor occurs in 33% of cases, and it was found that this immunomorphological profile significantly affects the occurrence of metastases, disease recurrence, and patient death, as well as that it determines the response to the ifosfamide-carbo-

svojoj studiji primetili da je povećana ekspresija *FGFR1* molekula među astrocitomima u pedijatrijskoj populaciji bila povezana sa uzrastom, lokacijom i gradusom tumora, dok je membranska ekspresija *pFGFR1* molekula bila povezana sa stepenom maligniteta i gradusom tumora [27].

Poznato je da *FGFR1* u neinvazivnim tumorima stimuliše rast i proliferaciju, dok u invazivnim tumorima stimuliše i proces migracije [32]. Interakcije između *FGFR1* i *NCAM* molekula opisane su prvi put u neuronima [16], a kasnije su opisane i u drugim tkivima [17,18]. Pokazano je da interakcija između ova dva molekula na membrani fibroblasta stimuliše migraciju ovih ćelija [28], dok je studija izvedena na eksperimentalnim ćelijskim kulturama epitelijalnog karcinoma jajnika ukazala na to da aktivacija *FGFR1* molekula *NCAM* molekulom povećava invazivnost tumorskih ćelija [19].

Ispitujući uticaj interakcija ova dva molekula na invazivnost karcinoma jajnika, Kolombo i Kalavaro su indukovali ekspresiju *NCAM* proteina u ćelijskim linijama koje su eksprimirale *FGFR1,* što je dovelo do transformisanja indolentnog karcinoma u invazivni. Međutim, indukcija ekspresije *NCAM* proteina modifikovanog tako da ne poseduje *FGFR1* vezujući domen, nije uticala na ponašanje karcinoma [33], čime je pokazano da su interakcije ova dva molekula, koje su do sada opisivane samo na površini ćelijske membrane, važne za agresivnost tumora. Pored mnogobrojnih karcinoma, u našoj studiji je koekspresija *NCAM* i *FGFR1* molekula detektovana i u svim onkocitomima. Međutim, obrazac ove koekspresije u benignim tumorima je bio isključivo sa citoplazmatskom lokalizacijom. Stoga bismo mogli reći da je, uprkos prisustvu oba molekula, u onkocitomima njihova interakcija izostala, jer je membranska imunopozitivnost bila isključivo karakteristika malignih tumora porekla bubrežnih ćelija.

Kolombo i Kalavaro su takođe otkrili da je koekspresija *NCAM* i *FGFR1* molekula bila najizraženija na ćelijama lokalizovanim na samoj periferiji tumora. Shvatili su to kao podatak koji govori u prilog hipotezi da koekspresija ovih molekula stimuliše migraciju i adheziju, procese ključne za metastaziranje tumora. Iako su kod najvećeg broja pacijenata u našoj studiji nedostajale informacije o prisustvu regionalnih i sistemskih metastaza tumora bubrega, ipak, analizirajući dostupne podatke nismo uočili povezanost između postojanja metastaza i membranske koekspresije *NCAM* i *FGFR1* molekula. Imajući u vidu značaj dimenzija tumora u određivanju stadijuma bolesti [34], mogli bismo pretpostaviti da je membranska koekspresija *NCAM* i *FGFR1* molekula imunomorfološki supstrat lokalnog tumorskog rasta. Tako je porast učestalosti membranske koekspresije ispitivanih proteina pratio porast T stadiju-

platin-etoposide (ICE) chemotherapy protocol [22]. In their study, Yang and Lu showed that co-expression of CCND1 and FGFR1 molecules exists in lung cancer, and that FGFR1 promotes EMT [21]. Examining pleuropulmonary blastoma cells, Shukrun and Golan observed that treatment with anti-NCAM immunoconjugate suppresses the growth of tumor cells in this tumor [23]. In their study, Egbivwie and Cockle observed that increased expression of FGFR1 among astrocytomas in the pediatric population was associated with age, location, and tumor grade, while membrane expression of pFGFR1 was associated with malignancy and tumor grade [27].

It is known that in non-invasive tumors FGFR1 stimulates growth and proliferation, while in invasive tumors it also stimulates the process of migration [32]. Interactions between FGFR1 and NCAM molecules were described for the first time in neurons [16], and were later described in other tissues [17,18]. It has been shown that the interaction between these two molecules on the membrane of fibroblasts stimulates the migration of these cells [28], while a study performed on experimental cell cultures of epithelial ovarian cancer indicated that the activation of the FGFR1 molecule by the NCAM molecule increases the invasiveness of tumor cells [19].

Examining the influence of the interaction between these two molecules on the invasiveness of ovarian cancer, Colombo and Callavaro induced the expression of the NCAM protein in cell lines expressing FGFR1, which led to the transformation of indolent cancer to invasive carcinoma. However, the induction of the expression of the NCAM protein, modified so that it does not possess the FGFR1 binding domain, did not affect the behavior of the cancer [33], thus showing that the interactions between these two molecules, which have been described thus far only on the surface of the cell membrane, are significant for tumor aggressiveness. In our study, in addition to numerous cancers, co-expression of NCAM and FGFR1 was also detected in all oncocytomas. However, the pattern of this co-expression in benign tumors was exclusively linked to cytoplasmic localization. Therefore, we could say that, despite the presence of both molecules, their interaction was absent in oncocytomas, because membrane immunopositivity was exclusively a characteristic of malignant tumors of renal cell origin.

Colombo and Callavaro also found that the co-expression of NCAM and FGFR1 was most pronounced in cells located at the very periphery of the tumor. They understood this to speak in favor of the hypothesis that the co-expression of these molecules stimulates migration and adhesion, processes crucial for tumor

ma tumora bubrega, što bi moglo indirektno da ukaže na veću sklonost *NCAM-FGFR1* pozitivnih tumora ka metastaziranju, budući da je TNM klasifikacija tumora važna, ne samo prilikom donošenja odluke o terapijskom pristupu, već ima uticaj i na prognozu bolesti i ukazuje na mogućnost metastaziranja [35].

Studija koju su sproveli Ronkainen i saradnici na različitim patohistološkim tipovima *RCC*-a, uključujući svetloćelijski, papilarni i hromofobni karcinom, ispitivala je ekspresiju *NCAM* molekula, pri čemu nije uočena korelacija između *NCAM* ekspresije i tipa, gradusa ili stadijuma tumora [10]. Činjenica da u ovoj studiji nije izučavana istovremena ekspresija drugih molekula, koji bi mogli da stupaju u interakcije sa *NCAM*-om u procesima bitnim za napredovanje tumora, mogla bi da objasni prividno neslaganje zaključaka ovog istraživanja i naših rezultata.

Danijel i saradnici su izneli tvrdnju da su *NCAM* pozitivni tumori agresivniji i da češće metastaziraju u nervna i neuroendokrina – *NCAM* pozitivna tkiva, poput nadbubrežne žlezde i centralnog nervnog sistema. Oni su opisali membransku ekspresiju u svetloćelijskom, ali ne i u papirilarnom i hromofobnom *RCC*-u [36]. Mi smo, s druge strane, u većini tumora istih patohistoloških tipova, uočili ne samo samostalnu *NCAM* ekspresiju, već i koekspresiju sa *FGFR1* molekulom.

Keresteš i Bunstra su odavno ukazali na potencijalni značaj nuklearne lokalizacije različitih receptora za faktore rasta. Ukazali su na činjenicu da prisustvo receptora i njihovih liganada u jedru dovodi do porasta proliferacije [37]. Čioni i Grous su, radeći na karcinomima dojke, otkrili da je granzim B odgovoran za dospevanje *FGFR*1 u jedro ćelije i da njegovim blokiranjem izostaje efekat aktivacije *FGFR1* molekula na proliferaciju, kao i to da *FGFR1* može da deluje kao transkripcioni faktor za neke gene odgovorne za proliferaciju [38]. Svi tumori bubrega u čijim smo jedrima detektovali *FGFR1* molekul pokazali su koekspresiju *FGFR1* i *NCAM* molekula na membrani. S obzirom na podatke o tome da *FGFR1* deluje kao transkripcioni faktor za neke gene, ne možemo isključiti mogućnost da je membranska ekspresija *NCAM* proteina rezultat ovakvog vida aktivnosti *FGFR1* molekula, ali to zahteva dodatna ispitivanja. Takođe, nije isključeno da je ekspresija *FGFR1* molekula u jedru odgovorna za napredovanje karcinoma bubrega, kao što je pokazano u tumorima dojke, mehanizmom koji ne podrazumeva interakcije sa *NCAM* proteinom [39].

Ispitivanja različitih molekularnih interakcija u patologiji tumora imaju za cilj definisanje ključnih procesa važnih u inicijaciji i progresiji rasta tumora, sposobnosti invazije u okolna tkiva i metastaziranju tumora. Nedavno je otkriveno da sintetska supstanca *PD173074*, koja predstavlja potentni *FGFR1* inhibitor,

metastases. For the majority of patients in our study information on the presence of regional and systemic metastases of renal tumors was lacking. However, analyzing the available data, we did not observe an association between the presence of metastases and membranous co-expression of NCAM and FGFR1. Bearing in mind the importance of tumor size in determining the stage of disease [34], we could assume that membranous co-expression of NCAM and FGFR1 is an immunomorphological substrate of local tumor growth. Thus, the increase in the frequency of membranous co-expression of the analyzed proteins was accompanied by an increase in the T stage of renal tumors, which could indirectly indicate a greater propensity of NCAM-FGFR1 positive tumors to metastasize, since the TNM classification of tumors is important, not only when deciding on a therapeutic approach, but it also has an impact on the prognosis of the disease and indicates the possibility of metastasis [35].

A study by Ronkainen et al. performed on different pathohistological types of RCC, including clear cell, papillary and chromophobe carcinoma, examined the expression of NCAM molecules. In this study, no correlation was observed between NCAM expression and tumor type, grade or stage [10]. The fact that the simultaneous expression of other molecules, which could interact with NCAM in processes essential for tumor progression, was not examined in this study could explain the apparent discrepancy between the conclusions of this study and our results.

Daniel et al. claimed that NCAM-positive tumors are more aggressive and that they more often metastasize to nervous and neuroendocrine – NCAM-positive tissues, such as the adrenal gland and the central nervous system. They described membranous expression in clear cell but not in papillary and chromophobe RCCs [36]. On the other hand, in most tumors of the same pathohistological types, we observed not only independent NCAM expression, but also co-expression with FGFR1.

Keresztes and Boonstra pointed out long ago the potential importance of the nuclear localization of different growth factor receptors. They highlighted the fact that the nuclear presence of receptors and their ligands leads to an increase in proliferation [37]. Chioni and Grose, working on breast cancers, discovered that granzyme B is responsible for the delivery of FGFR1 to the cell nucleus and that blocking it eliminates the effect of FGFR1 activation on proliferation, as well as that FGFR1 can act as a transcription factor for some genes responsible for proliferation [38]. All renal tumors in whose nuclei we detected FGFR1 showed co-expression of FGFR1 and NCAM molecules on the membrane.

sprečava proliferaciju tumorskih ćelija i indukuje promenu njihove morfologije putem indukcije mezenhimno-epitelne transformacije, redukujući invazivnost i rast tumora [40,41]. S obzirom na iznete zaključke o uticaju interakcije *NCAM* i *FGFR1* molekula, u daljim istraživanjima moglo bi se otvoriti pitanje mogućnosti primene *FGFR1* inhibitora (*PD173074*) u cilju usporavanja lokalnog rasta tumora bubrega i redukcije njegovog invazivnog potencijala.

ZAKLJUČAK

Moguće je da samo membranska koekspresija *NCAM* i *FGFR1* molekula imaju uticaja na agresivnije biološko ponašanje tumora, utičući tako i na stadijum bolesti, te je samo ovakav obrazac koekspresije značajan za procenu njihove biološke aktivnosti, koja nije uočena ni kod jednog benignog tumora, uprkos prisustvu citoplazmatske koekspresije. Postoji mogućnost da je kod manjeg broja pacijenata fenomen membranske *NCAM-FGFR1* koekspresije rezultat prisustva *FGFR1* molekula u jedru tumorskih ćelija. Potrebno je sprovesti dalja ispitivanja, na većem broju pacijenata, kako bi se detaljno ispitao značaj koekspresije *NCAM* i *FGFR1* molekula kod pacijenata sa tumorima bubrega.

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Given the data that FGFR1 acts as a transcription factor for some genes, we cannot rule out the possibility that membranous expression of the NCAM protein is the result of this kind of FGFR1 activity, but this requires additional research. Also, it is possible that the expression of FGFR1 in the nucleus is responsible for the progression of renal cancer, as shown in breast tumors, by a mechanism that does not involve interactions with the NCAM protein [39].

Investigations of various molecular interactions in tumor pathology aim to define the key processes important in the initiation and progression of tumor growth, the ability to invade surrounding tissues, and tumor metastasis. It was recently discovered that the synthetic substance PD173074, which is a potent FGFR1 inhibitor, prevents the proliferation of tumor cells and induces a change in their morphology through the induction of mesenchymal-epithelial transformation, reducing invasiveness and tumor growth [40,41]. Considering the above conclusions about the effect of the NCAM-FGFR1 interaction, further research could open the question of the possibility of applying the FGFR1 inhibitor (PD173074) in order to slow down the local growth of renal tumors and reduce their invasive potential.

CONCLUSION

It is possible that only membranous co-expression of NCAM and FGFR1 affects more aggressive biological behavior of a tumor, thus affecting the stage of the disease, and that only this pattern of co-expression is significant for the assessment of their biological activity, which was not observed in any benign tumor, despite the presence of cytoplasmic co-expression. It is possible that in a smaller number of patients the phenomenon of membranous NCAM-FGFR1 co-expression is the result of the presence of FGFR1 molecules in the nucleus of the tumor cells. It is necessary to perform further studies, on a larger number of patients, in order to make a detailed analysis of the significance of the co-expression of NCAM and FGFR1 in patients with renal tumors.

Conflict of interest: None declared.

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