Comparison of expression pattern of monoamine oxidase A with histopathologic subtypes and tumour grade of renal cell carcinoma

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Studies of the biochemical properties of MAO-A (monoamine oxidase) are numerous, but the information about determination of MAO-A in human normal and tumour renal tissue is limited. Our objectives in the present study were to determine the localization of MAO-A in normal kidney and level of expression of this protein in tumour kidney.

Material/Methods: Enzyme immunohistochemical method was chosen for detection of MAO-A in 63 clinical samples of all histopathological types of RCC (renal cell carcinoma). Our results were compared to basic clinical and histopathological parameters such as histopathological type and tumour grade. We also compared MAO-A expression between normal and tumour tissue samples.

Results: We confirmed the elevated expression of MAO-A in high-grade tumours of renal cell carcinoma specimens. The percentage of MAO-positive samples progressively increased from 9% in grade 2 to 45% in grade 3. We also noted high levels of MAO-A immunoreactivity in epithelial cells of proximal tubules in normal renal tissue. MAO-A was absent or very low in epithelial cells of distal tubules and glomerular capsule, as well as in endothelial cells of renal vessels.

Conclusions: Taken together, our results and findings of other studies show that MAO-A expression in high-grade tumours may have a direct role in maintaining a dedifferentiated phenotype and promoting aggressive behaviour. The ability of clorgyline (an MAO-A inhibitor) to counteract oncogenic pathways and promote differentiation suggests that MAO-A inhibitors, which have been used for many years in clinical practise for treating neurological disorders, could be therapeutic options for advanced stages of tumours.

key words: **monoamine oxidase • renal cell carcinoma • immunohistochemical expression**

BACKGROUND

Identifying consistent changes in cellular function that occur in multiple types of cancer could revolutionize the way cancer is treated. Previous work has produced promising results such as the identification of a mutation in p53, which is a protein responsible for repairing cellular DNA, occurring in approximately 50% of all cancers [1]. The discovery of similarities among various cancer tissues is the first step in identifying a common mechanism that contributes to the development of cancer. Once a change is identified, appropriate therapeutic targets can be developed to help physicians identify at-risk individuals and improve patient care. Indeed, novel therapeutic strategies have been developed as a result of the extensive study of p53 [2].

Overall, little is known about the function of MAOs in cancer [3]. MAOs are flavoprotein enzymes located in the mitochondrial outer membranes that are widely distributed among all living organisms. They are key regulatory and protective enzymes because their substrates include biogenic monoamines such as neurotransmitters, drugs and dietary amines. The enzymes exist in 2 forms, MAO-A and MAO-B, which are different gene products and have different substrate specificities. MAO-A preferentially degrades norepinephrine (NE) and serotonin, whereas MAO-B has a greater affinity for phenylethylamine and benzylamine. Dopamine is a common substrate of both MAO-A and MAO-B [4]. The 2 MAO isoforms can also be differentiated according to their inhibition by synthetic compounds. MAO-A is selectively inhibited by clorgyline, whereas MAO-B is selectively inhibited by L-deprenyl. MAO-A has been widely studied in the context of neurological disorders such as depression and Parkinson's disease [5]. MAO-A is also expressed in non-neuronal tissues, but its function in these tissues is unknown. The enzymes have been identified outside the central nervous system in organs such as liver, kidney and intestines [6]. Recently, MAO-A was found to be one of the most highly overexpressed genes in high-grade prostate cancer (2.4-fold higher in Gleason grade 4/5 than in grade 3). Immunohistochemical evaluation of tissue prostate samples confirmed that MAO-A was also overexpressed at the protein level in grade 4/5 cancers [7]. Because the progression of prostate cancer from grade 3 to 4/5 marks a critical change from curable to lethal [8,9], increased expression of MAO-A in grade 4/5 cancer raises the possibility that activity of this enzyme is a key factor in the increased lethality of high-grade of prostate cancer.

Studies of the biochemical properties of MAO-A are numerous, but the information about determination of MAO-A in human normal and tumour renal tissue is limited. Our objectives in the present study were to determine the localization of MAO-A in normal kidneys and level of expression of this protein in tumour kidneys. The acquired results should help us to clarify and better understand the possible involvement of MAO-A in the pathogenesis (process of carcinogenesis) and its role as a prognostic factor of renal cell carcinoma.

MATERIAL AND METHODS

Patients

The goal of this study was immunohistochemical evaluation of MAO-A in 63 samples of renal cell carcinoma (RCC). Our

Table 1. Characteristic of 63 patients with renal cell carcinoma.

Features		No of cases (%)	
All patients		63	
Sex	female	26	(41%)
	male	37	(59%)
Age	≤50	12	(19%)
	≥51	51	(81%)
Histo-pathol. type	RCC – clear-cell type	51	(81%)
	RCC – papillary type	5	(8%)
	RCC – chromophobe type	3	(5%)
	RCC – multilocular cystic type	1	(1.5%)
	RCC – sarcomatoid type	1	(1.5%)
	RCC – unclassified type	2	(3%)
Nuclear grade (Fuhrman)	1	20	(32%)
	2	32	(51%)
	3	11	(17%)
MAO expression	Positive	11	(17%)
	Negative	52	(83%)

results were compared to basic clinical and histopathological parameters such as histopathological type and tumour grade. We also compared MAO-A expression between normal and tumour tissue samples. The samples were obtained from the Department of Pathology, Pasteur Faculty Hospital, Košice, Slovak Republic. Patients and tumour characteristics are summarized in Table 1. Our renal cell carcinoma samples were divided according to histopathological type into 2 groups: the first group was conventional type RCC (clear cell type) =51 samples; the second group was other types of RCC (5 papillary types, 3 chromophobe, 1 sarcomatoid type, 1 multilocular cystic type and 2 unclassified types) =12 samples. For immunohistochemical detection of MAO-A, rabbit polyclonal primary antibody was used (H-70) (Santa Cruz Biotechnology, Inc.).

Immunohistochemical detection of MAO-A

After paraffin removal, sections were finally washed in phosphate-buffered saline containing 0.05% Tween-20 (PBS-Tw), pH 7.6. Endogenous peroxidase activity was blocked by 0.3% H_2O_2 in methanol for 10 minutes at room temperature. MAO-A staining procedure continued by blocking nonspecific staining with normal blocking serum (1.5% blocking serum in PBS) for 30 minutes at room temperature. The next step was application of primary antibody, which was applied overnight in a humidified chamber at 4°C. After rinsing in PBS-Tw (3×5 minutes) the sections were subsequently incubated with the secondary antibody,
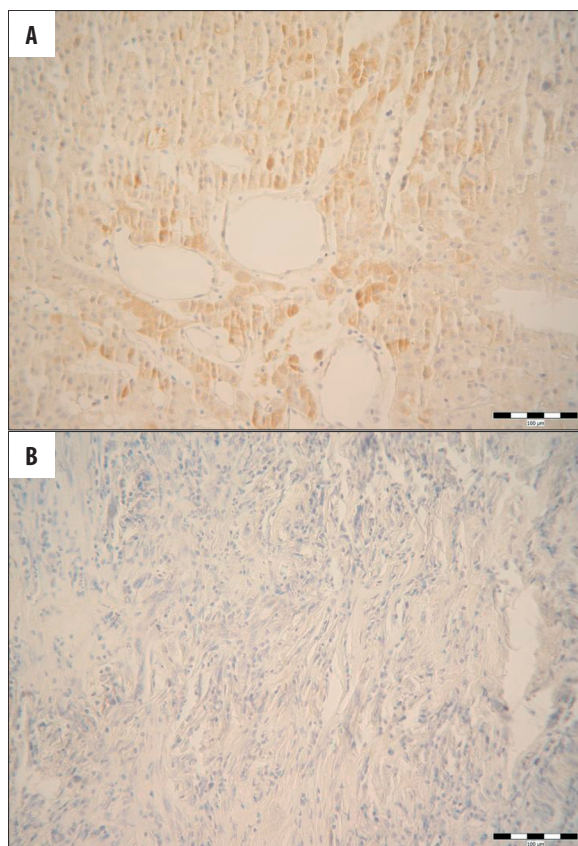
Figure 1. Renal cell carcinoma-other type with MAO-A positivity (**A**). Negative expression of MAO-A, note complete absence of MAO-A immunostaining in carcinoma cells of RCC-other type (**B**).

prediluted biotinylated secondary antibody (ABC Staining System, Santa Cruz Biotechnology, Inc.), for 30 minutes at room temperature. The slides were washed with PBS-Tw and submitted to application of AB enzyme reagent: avidin and biotinylated horseradish peroxidase (Santa Cruz Biotechnology, Inc.) for 30 minutes at room temperature. The sections were visualized with DAB (3.3'-diaminobenzidine tetrahydrochloride) at a concentration of 0.5 mg/ml in Tris buffer (pH 7.6) and 0.015% H_2O_2. Slides were stream-rinsed with tap water, counterstained with hematoxylin for 2 minutes, washed in tap water, dried, mounted and cover slipped. The control of immunohistochemical procedure was made by omitting the primary antibody during the process of immunohistochemical staining. Results of the immunostaining were evaluated by light microscopy. The slides were examined independently by 2 observers blinded to patient characteristics. Expression of MAO A was quantified using a visual grading system based on number/percentage of positive tumour cells graded on a scale 0 to 3+: 0% of positive cells = negative; 1–10% = 1+; 11–90% = 2+; 91–100% = 3+. Considered as positive were only 2+ and 3+ (over 10%) samples, whereas 0 and 1+ were regarded as negative (less than 10%). To evaluate the statistical significance between MAO-A protein expression and histopathological type and tumour grade, the chi square test and Kruskal-Wallis test were used. P<0.05 was considered to be significant.
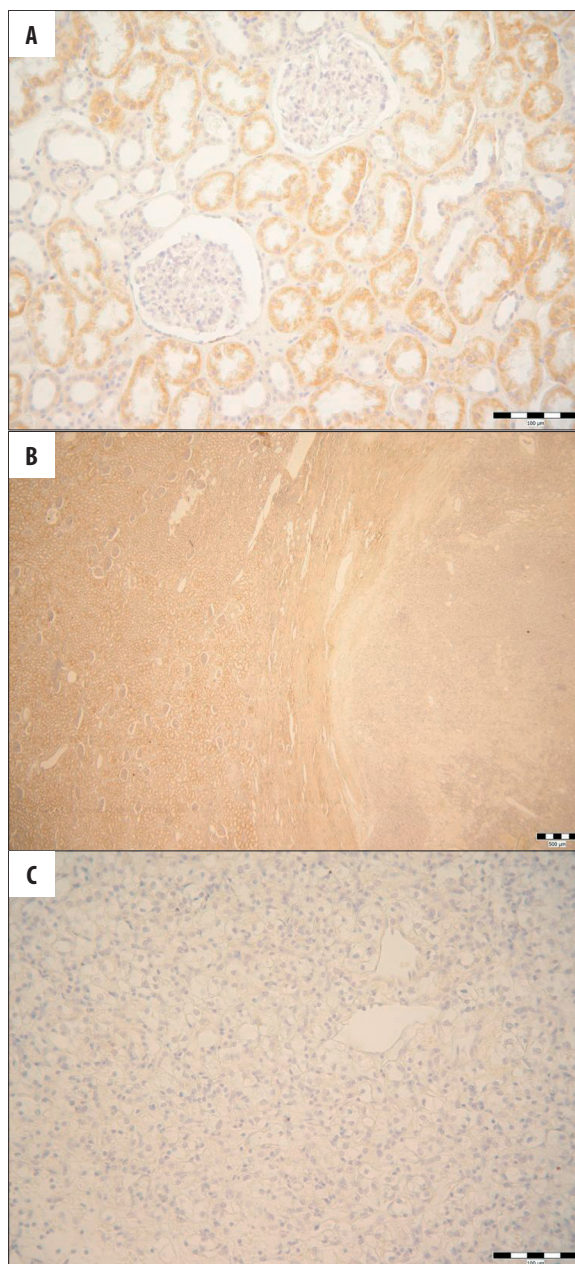

Figure 2. The boundary line (**B**) of normal renal tissue with presence of MAO-A (**A**) and malign renal tissue with no expression of MAO-A (**C**).

RESULTS

Immunohistochemical MAO-A expression in normal renal tissue and renal cell carcinoma

Strong brown cytoplasmic staining was visible in MAO-A-positive cells. In the normal renal tissue (control group) we observed strongly positive intracellular expression of MAO-A protein in epithelial cells of proximal tubules (Figure 1A).

Ten (19%) tissue samples of the conventional type of RCC were MAO-A-positive (Figure 2A, B). As regards other types of RCC (n=12), only 1 (8%) sample was MAO-A-positive (Figure 3A). No nuclear reaction for MAO-A protein was
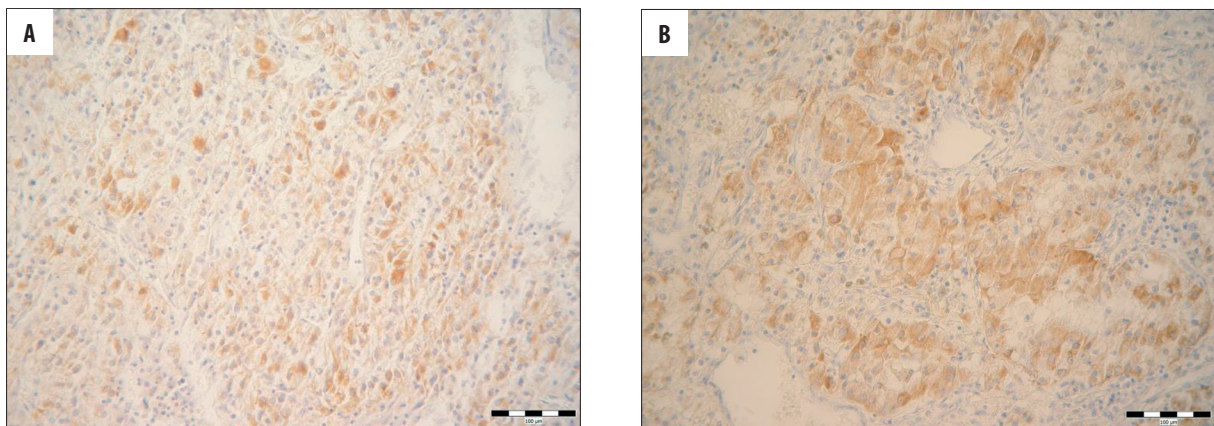
Figure 3. Strong MAO-A immunoreactivity with brown cytoplasmic staining in tumor cell of RCC-clear cell type, grade 1 (**A**) and in grade 3 (**B**).

Table 2. Levels of MAO-A expression in 63 samples of renal cell carcinoma: 51 cases of clear-cell RCC and 12 cases of other type RCC.

Expression of MAO (%)*	Neg	1+	2+	3+	No. negative samples	No. positive samples
RCC – clear-cell type (n=51)	36 (71%)	5 (10%)	7 (13%)	3 (%)	41 (81%)	10 (19%)
RCC – other type (n=12)	9 (75%)	2 (17%)	1 (8%)	0 (0%)	11 (92%)	1 (8%)

0% of positive cell = negative; 1–10% = 1+; 11–90% = 2+; 91–100% = 3+. Chi square test, p>0.05.

revealed in any of the analyzed cases. No immunoreactivity was seen in negative MAO-A samples (Figure 3B, 1C).

Comparison of MAO-A expression with histopathologic subtypes

We did not find a statistically significant difference in MAO-A overexpression among the histopathologic subtypes of RCC (p>0.05). More details concerning the expression of MAO-A are illustrated in Table 2.

Comparison of MAO-A expression with tumour grade of RCC

Tumour grade, a clinically relevant predictor parameter, was determined and compared with expression. The comparison of this parameter was evaluated in 63 clinical samples of all histopathological types of RCC.

Statistically significant differences in MAO-A expression in renal cell carcinomas were observed with respect to the tumour grade (p<0.05, p=0.0221). In grade 1 (n=20), 3 (15%) tissue samples was MAO-A positive. Three (9%) tumours were positive in grade 2 (n=32) and 5 (45%) MAO-A positive specimens were grade 3 (n=11). The exact numbers of tumour tissue samples and grades are shown in Figure 4.

Discussion

Renal cell carcinoma (RCC) is the most common epithelial malignancy of the kidneys in adults, representing over 90 percent of the primary renal neoplasms [11]. The incidence of renal cell carcinoma has been increasing steadily
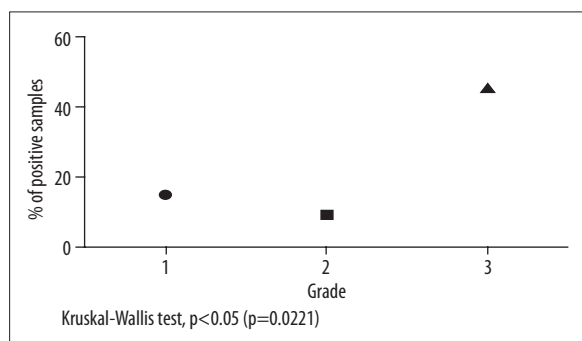
Figure 4. Statistical analysis between grade and% of MAO-A positive samples.

over recent decades. The worldwide incidence and mortality rate is about 270 000 cases and 120 000 deaths, respectively [12]. Considering renal cell carcinoma, there are still controversies about the use of particular markers as prognostic factors.

Recent evidence supports a longstanding hypothesis that neurotransmitters, such as catecholamines and neuropeptides, can influence tumour growth and progression. Norepinephrine and epinephrine are potent stimulators of vascularization, acting both by inducing the release of angiogenic factors from tumour cells and directly on endothelial cell functions. Dopamine, on the other hand, interferes with VEGF signalling in endothelial cells, blocks its angiogenic functions, and inhibits tumour growth. Another neurotransmitter, neuropeptide Y, co-released with norepinephrine, directly stimulates angiogenesis. Depending on

the neurotransmitter and type of tumour, these effects can be both stimulatory and inhibitory [10].

The role of monoamino oxidase in the pathogenesis of RCC and its role as a prognostic factor in RCC remain doubtful. Monoamino oxidases are the major enzymes involved in the elimination of biogenic amines by their oxidation. The crucial role of these enzymes in tumour progression is the elimination of amines necessary for cell growth [13]. Biogenic amines could be considered as both poisons and protectors [14]. Their oxidation products, H_2O_2 (and therefore reactive oxygen species, ROS) and amino aldehydes, are considered cell growth inhibitors. Tumour initiation in hamster kidneys has been related to a significant increase in the production of H_2O_2 and hydroxyl radical through estrogen receptor-mediated activation of MAO [15]. The findings of True et al. [7] prompted us to investigate the level of MAO-A in renal cell carcinoma. We confirmed the elevated expression of MAO-A in high-grade tumours of renal cell carcinoma specimens. The percentage of MAO-positive samples progressively increased from 9% in grade 2 to 45% in grade 3. We also noted high levels of MAO-A immunoreactivity in epithelial cells of proximal tubules in normal renal tissue. MAO-A was absent or very low in epithelial cells of distal tubules and glomerular capsule, as well as in endothelial cells of renal vessels.

Elevated expression of MAO-A was confirmed in high grade prostate cancers [16]. Authors also noted high levels of MAO-A immunoreactivity in the basal epithelia of normal prostatic glands. MAO-A was absent or very low, however, in normal differentiated luminal epithelia. Primary cultures of normal basal epithelial cells retained high expression of MAO-A. Treatment with clorgyline, an irreversible MAO-A inhibitor, induced secretory cell-like morphology, and induced mRNA and protein expression of androgen receptor, the quintessential characteristic of prostatic secretory epithelial cells. These results suggest that the function of MAO-A in normal prostatic epithelium is to maintain basal or progenitor cells in the undifferentiated state. A similar function was reported in neuronal stem cells, in which inhibition of MAO-A promotes stem cell differentiation [17]. Based on these findings in normal cells, one can hypothesize that elevated expression of MAO-A in high-grade cancers contributes to their poorly differentiated phenotype. Microarray analysis of primary cultures derived from high-grade prostate cancers treated with clorgyline showed induction of many genes associated with secretory cell differentiation and inhibition of many gene pathways associated with oncogenesis [18].

Conclusions

Based on our findings in normal cells, we hypothesize that elevated expression of MAO-A in high-grade RCC contributes to its poorly differentiated phenotype. Taken together, these recent results suggest that MAO-A expression in high-grade tumours may play a key role in maintaining a dedifferentiated phenotype and promoting aggressive behaviour.

The ability of clorgyline to counteract oncogenic pathways and promote differentiation suggests that MAO-A inhibitors, used for many years in clinical practice for treating neurological disorders, could be therapeutic options for advanced stages of tumours. They have the ability to prevent progression of well differentiated to poorly differentiated cancers, and have anti-tumour activity. To our knowledge this is the first paper describing expression of MAO-A and its relationship with grade in kidney cancers.

Abbreviations

MAO-A – monoamine oxidase A; RCC – renal cell carcinoma; VEGF – vascular endothelial growth factor.

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