Ассоциация экспрессии генов рецепторов фактора роста тромбоцитов альфа и бета (*PDGFRA* и *PDGFRB*) с биохимическим рецидивом рака предстательной железы после радикальной простатэктомии

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Проведен метаанализ результатов профилирования транскриптома образцов рака предстательной железы после радикальной простатэктомии у пациентов без метастатического поражения лимфатических узлов. Показана взаимосвязь экспрессии рецепторов фактора роста тромбоцитов альфа и бета (PDGFRA и PDGFRB), ассоциированных с лимфогенным метастазированием, с вероятностью и временем наступления биохимического рецидива.

Ключевые слова: *рак предстательной железы, лимфатический узел, метод опорных векторов, биохимический рецидив*

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Expression of platelet-derived growth factor alpha and beta genes *PDGFRA* and *PDGFRB* associated with biochemical recurrence of prostate cancer after radical prostatectomy

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We performed genome-wide transcriptome meta-analysis of prostate cancer samples after radical prostatectomy of patients without lymph node metastasis. Significant associations were determined between expression of platelet-derived growth factor alpha and beta genes (PDGFRA and PDGFRB) and probability and time of onset of biochemical recurrence.

Key words: *prostate cancer, lymph node, support vector machine, biochemical recurrence*

Lymph node (LN) metastasis in prostate cancer (PC) is considered an unfavorable prognostic factor [1]. Approximately 75% of patients with LN metastases develop bone metastases within five years regardless of treatment [2].

The high rate of false-negative histological findings in LN metastases is primarily associated with inability to perform comprehensive examination of LNs [3]. Extensive assessment of LNs using serial step sectioning and immunohistochemical staining increases the detection rate of LN metastases and/or isolated tumor cells (TCs). However, this approach is associated with additional expenses and relatively small increase in diagnostic accuracy [4].

Metastasis is a complex process that involves TCs escaping from the primary tumor, entering the systemic circulation, and invading distant organs (usually liver or lungs) or LNs, resulting in the establishment of new tumors. Several mechanisms of TC dissemination are currently being discussed: invasion into surrounding tissues, metastasis to distant organs through the tumor-induced capillary network, and spreading to regional LNs and then to distant LNs (with subsequent invasion to distant organs) through tumor-associated lymphatic vessels. Despite the clinical importance of LN metastasis [1], the molecular mechanisms underlying the dissemination of TCs to regional and distant LNs through lymphatics remain poorly understood. Traditionally, lymphatics have been assigned a passive role in LN metastasis. TCs are believed to be carried into the lymphatics with the interstitial fluid [5] due to their higher permeability compared to blood capillaries and the absence of a continuous basal membrane [6]. The discovery of vascular endothelial growth factors C and D (VEGF-C and VEGF-D) and their VEGFR-3 receptor on the surface of lymphatic endothelial cells allowed to revise the mechanisms underlying lymphogenic metastasis [7]. VEGF-C and VEGF-D were shown to be involved in tumor-induced lymphangiogenesis (in xenografts and mouse tumor models) and ultimately promote regional LN metastasis [8–11]. The expression of VEGF-C in PC cells

correlates with LN metastasis. Moreover, high VEGF-C expression in PC cells is associated with increased lymphatic vessel density in the surrounding tumor stroma [11]. However, downregulation of both VEGF-C and VEGFR-3 expression in a mouse model of human PC leads to a more than 10-fold decrease in lymphatic vessel density in the stroma, but does not reduce the number of LN metastases. This finding indicates that pre-existing lymphatics are enough for lymphatic tumor dissemination [12].

Objective: to assess the correlation between the expression of genes associated with lymphogenous metastasis and biochemical recurrence (BCR) of PC in patients after radical prostatectomy without LN involvement.

Materials and methods

We assessed cumulative prognostic value of genes associated with lymphangiogenesis using support vector machine (SVM) analysis [13]. Using publicly available microarray data sets, we generated a metasample that included the GSE46 602 data set [14] as a training sample and the GSE10 645 [15] data set as a control sample. Affymetrix GeneChip Human Genome U133 Plus 2.0 Array was used to evaluate levels of gene expression in the training sample, whereas in the control sample it was assessed using the Illumina DASL Assay. None of the patients in the training sample had LN metastases. In the test sample, the proportion of individuals with LN metastases was < 20% and did not differ among patients with BCR and without BCR. Clinical characteristics of patients are shown in Table 1.

Таблица 1. *Клинические характеристики пациентов*
Table 1. *Clinical characteristics of the patients*

Характеристика Characteristic	Обучающая выборка Training sample		Контрольная выборка Control sample	
	без БХР (n = 14) without BR (n = 14)	с БХР (n = 22) with BR (n = 22)	без БХР (n = 195) without BR (n = 195)	с БХР (n = 200) with BR (n = 200)
Возраст, лет Age, years	61,9 ± 5,2	62,4 ± 6,3	65,3 ± 6,4	65,6 ± 6,4
Период наблюдения/время наступления БХР, лет Follow-up duration/time of BR, years	5,0 ± 1,6	2,0 ± 1,9	11,6 ± 3,1	2,7 ± 2,1
Предоперационный уровень простатического специфического антигена, нг/мл Preoperative prostate-specific antigen level, ng/ml	12,0 ± 5,1	22,1 ± 10,6	11,9 ± 13,3	18,8 ± 22,5
Сумма баллов по шкале Глисона: Total Gleeson score:				
4	1	0	2	7
5	5	2	44	72
6	6	3	110	27
7	1	14	16	87
8	0	3	22	7
9	1	0	1	7
10	0	0	2	72
Стадия: Stage:				
pT2a	3	0	111	54
pT2c	10	6	111	54
pT3a	1	7	43	45
pT3b	0	9	20	54
$pTxN^+$	0	0	21	47
Край резекции: Resection margin:				
отрицательный negative	12	8	н/д n/a	н/д n/a
положительный positive	2	14	н/д n/a	н/д n/a

Примечание. *БХР – биохимический рецидив; н/д – нет данных.*
Note. *BR – biochemical recurrence; n/a – data not available.*

Таблица 2. *Статистические характеристики лучших классификаторов в обучающей выборке*
Table 2. *Statistic characteristics of the best classifiers in the training sample*

Ген 1 Gene 1	Ген 2 Gene 2	Чувствительность Sensitivity	Специфичность Specificity	AUC
PDGFRB	*IGF2*	0,71	0,95	0,89
PDGFRB	*TEK*	0,86	0,86	0,89
PDGFRB	*FLT4*	0,86	0,86	0,87
PDGFB	*IGF1R*	0,86	0,86	0,87
PDGFRB	*PDGFRA*	0,50	0,95	0,87
PDGFRB	*PDGFB*	0,64	0,91	0,87
PDGFRB	*IGF1R*	0,79	0,86	0,86
PDGFRB	*IGF1*	0,71	0,86	0,86
PDGFRB	*KDR*	0,64	0,86	0,86
IGF1R	*TEK*	0,79	0,82	0,84
FLT4	*IGF1R*	0,64	0,86	0,82
PDGFB	*FLT4*	0,64	0,77	0,82
KDR	*IGF1R*	0,36	0,95	0,80
PDGFB	*IGF2*	0,43	0,95	0,80

Примечание. *AUC – площадь под ROC-кривой.*
Note. *AUC – area under the ROC-curve.*

The list of lymphogenous metastasis-associated genes was based on the analysis of previous publications and included the following genes: VEGFC, VEGFD, VEGFR3, VEGFR2, NRP2, VEGFA, NRP1, IGF1, IGF2, IGF1R, HGF, KIT, ANGPT1, ANGPT2, TEK, IL7, IL7R, EFNB2, EFNB4, PDGFB, PDGFRA, PDGFRB, GH1, GHR, ADM, and FGFR3.

To assess cumulative prognostic value of gene pairs, we constructed a binary linear SVM classifier on the training sample [21] (see more details in [13]). We used the area under the receiver operating characteristics (ROC) curve (AUC) to evaluate the performance of the classifier. The performance of the classifier in the training sample was considered satisfactory if the AUC is 0.8 or greater, in the control sample–0.6 and greater.

At the final stage of the analysis, we evaluated classifier performance in the test sample, which was not used in constructing the SVM classifier. For the test sample, we plotted Kaplan–Meier survival curves and assessed their divergence using log-rank test (p-values are two-sided).

Results

Analyzing the discriminative ability of each individual gene in the training sample, we found that only PDGFRB gene had AUC > 0.8 (sensitivity 0.77; specificity 0.93). Building a gene-pair classifier allowed to identify 14 gene pairs with AUC > 0.8. The most informative gene pairs are shown in Table 2.

The analysis of the control sample demonstrated rather low discriminative ability of lymphangiogenesis-associated genes. The expression of the PDGFRA gene was found to have the highest discriminative power in predicting BCR (sensitivity 0.5; specificity 0.7; AUC 0.61) (Figure 1).

Two out of 14 gene pairs from the training sample had AUC > 0.6. Four gene pairs demonstrated significant divergence of Kaplan-Meier curves for relapse-free survival (Table 3, Figure 2).

Discussion

Among all lymphangiogenesis-associated genes, the highest discriminative ability for BCR in the training sample was demonstrated by the PDGFRB gene. However, in the testing sample, this gene showed the lowest discriminative power, whereas the PDGFRA gene was the most informative. The receptors encoded by these genes as well as their ligands play a key role in regulating cell growth and proliferation and have a significant impact on cancer cells and tumor environments. In PC, PDGF-D is likely to be involved in osteoclastic differentiation and establishment of bone metastasis. High levels of PDGFR-B in the tumor

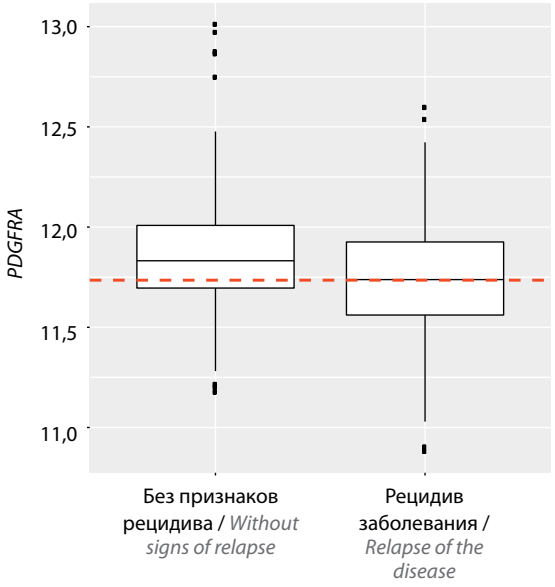

Рис. 1. *Экспрессия гена PDGFRA в контрольной выборке*
Fig. 1. *PDGFRA gene expression in the control sample*

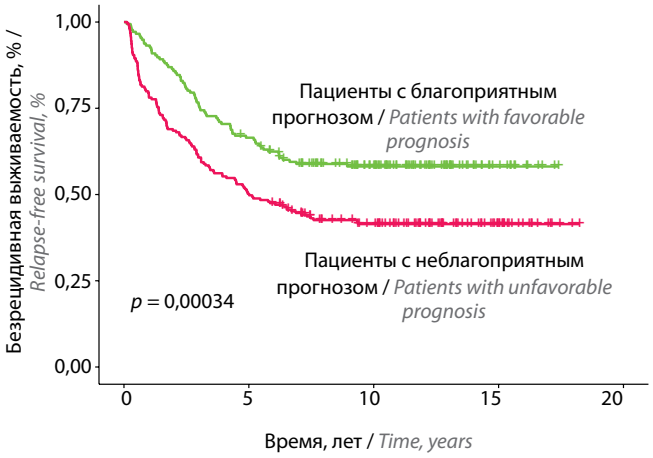

Рис. 2. *Кривые Каплана–Майера, построенные на основании экспрессии генов PDGFRA и PDGFRB в образцах контрольной выборки*
Fig. 2. *Kaplan–Meier curves plotted based on PDGFRA and PDGFRB genes expression in the samples of the control sample*

Таблица 3. *Статистические характеристики лучших классификаторов в контрольной выборке*
Table 3. *Statistical characteristics of the best classifiers in the control sample*

Ген 1 Gene 1	Ген 2 Gene 2	Чувствительность Sensitivity	Специфичность Specificity	AUC	*p* для кривых Каплана–Майера *p* for the Kaplan–Meier curves
PDGFRA	*PDGFRB*	0,53	0,64	0,63	0,0003
PDGFRB	*IGF1*	0,58	0,61	0,60	0,0002
PDGFB	*FLT4*	0,60	0,53	0,60	0,01
IGF1R	*PDGFB*	0,69	0,44	0,58	0,01
PDGFB	*IGF2*	0,65	0,46	0,57	0,05
IGF1R	*FLT4*	0,62	0,49	0,56	0,04

stroma and non-tumor prostate tissue in PC were associated with shorter tumor-specific survival [23]. Although the use PDGFR inhibitors did not improve survival of patients with PC (and in some cases even triggered the development of advanced PC) [24], we should not entirely ignore these markers for choosing an appropriate treatment strategy for patients with PC.

Nordby et al. investigated the prognostic impact of PDGFR-B and its ligands (PDGF-B and PDGF-D) in a cohort of 535 patients after prostatectomy. The expression of ligands PDGF-B and PDGF-D was assessed in neoplastic tissue and tumor-associated stroma. The expression of PDGFR-B was observed in benign hyperplastic tissue and tumor-associated stroma, but not in epithelium. High stromal expression of PDGFR-B was associated with clinical relapse (hazard ratio (HR) = 2.17, p = 0.010) and BCR (HR = 1.58, p = 0.002) [25]. However, our results suggest that simultaneous evaluation of PDGFRA and PDGFRB expression ensures higher AUC values than the analysis of individual gene expression. In a two-variable model, higher PDGFRB expression and lower PDGFRA expression correlate with a higher risk of relapse. Despite the fact that both receptors are involved in the transduction of mitogenic signals, PDGFR-B seems to be much more closely associated with cell transformation than PDGFR-A [26]. Since PDGFR-A and PDGFR-B can form homo- and heterodimeric complexes when conducting signals from their ligands, higher expression of PDGFRB along with lower expression PDGFRA probably ensures the formation of a larger number of homodimeric PDGFR-B due to their enhanced transformation potential, which presumably explains the observed predictive trends in this gene pair.

The PDGFRB/IGF1 gene pair also demonstrated high discriminative power in predicting BCR. Trevino et al. has earlier described probable interaction between IGF1 and PDGF-BB in PC [27]. The IGF1 gene encodes the insulin-like growth factor 1, which signaling pathway is associated with glucose metabolism and homeostasis, cell growth, proliferation and differentiation, and apoptosis. Moreover, the IGF axis is likely to be involved in the development of certain tumors, including PC [28].

It was found that several single nucleotide polymorphisms in the IGF1 gene correlated with more advanced PC and higher risk of BCR [29]. Patients with Gleason score (GS) 3 + 4 PC demonstrated significantly increased expression of the IGF1 receptor (IGF1R) in cancerous versus benign tissue; however, in patients with GS 5, the difference was non-significant. Furthermore, both IGF1R and its ligand (IGF1) failed to predict biochemical recurrence in PC [28]. However, this study analyzed the levels of IGF1 and IGF1R proteins, whereas we evaluated levels of gene expression by measuring messenger RNAs. In the testing sample, the IGF1 gene was included into a gene pair with AUC > 0.6. Higher levels of PDGFRB expression and lower levels of IGF1 expression were associated with an increased risk of relapse. Simultaneous changes in the expression of these genes probably lead to certain changes in activation of signaling pathways and result in BCR.

Conclusion

LN metastasis is an unfavorable prognostic factor in PC. Histological examination of LNs is associated with a high probability of false-negative results. Our findings suggest a correlation between the expression of lymphogenous metastasis-associated genes PDGFRA and PDGFRB and the probability and time of PC BCR in patients without LN involvement after radical prostatectomy.

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