P73: STUDYING THE IMMUNE MICROENVIRONMENT FAVOURING INITIATION OF INVASION IN CERVICAL CARCINOMA

Olga V. Kurmyshkina[1*], Pavel I. Kovchur[1,2], Tatyana O. Volkova[1]

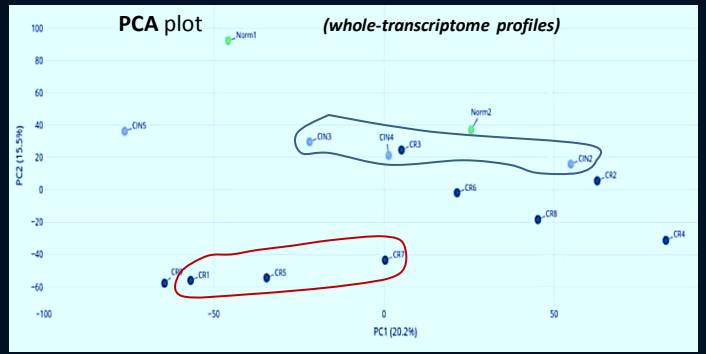
* olya.kurmyshkina@yandex.ru

[1] Institute of High-Tech Biomedicine, Petrozavodsk State University, Russia
[2] the Republican Oncologic Dispensary, Petrozavodsk, Russia

Background: To date, a rather substantial body of evidence has accumulated indicating that cervical cancer is immunologically heterogeneous and comprises several molecular immunophenotypes. A significant proportion of cervical cancers show enrichment in intratumoral immune cells, *inflammatory microenvironment, high level of interferon signaling, accompanied by up-regulation of immune checkpoints and immune suppression*. However, not much is known about whether such patterns are detectable in the *early* stages of cervical cancer progression, during the *transition from intraepithelial neoplasia to early-stage invasive* carcinoma.

Methods:
RNA-sequencing and bioinformatics pathway analysis, gene co-expression network and PPI network analysis were performed using a panel of 16 surgical samples which included non-dysplastic cervical epithelium, cervical intraepithelial neoplasia (CIN), carcinoma *in situ* (CIS), micro-carcinoma (FIGO stage IA1), stages IA2–IIB of invasive squamous carcinoma of the cervix:

Sample ID	Degree / Stage
Norm_1	HPV(- morphologically normal cervical epithelium
Norm_2	HPV(+) morphologically normal cervical epithelium
CIN_1	**CIN3**
CIN_2	**CIN3 (CIS)**
CIN_3	**CIN2/3**
CIN_4	CIN3 (CIS)
CIN_5	CIN1
CR_1	**IA1**
CR_2	IB1
CR_3	IA1
CR_4	IA1
CR_5	**IB1**
CR_6	IB2/IIA1
CR_7	**IIB**
CR_8	IA1
CR_9	IA2



RESULTS

A total of 151 significant differentially expressed genes (DEGs) were found between early-stage invasive cancer and pre-invasive high-grade neoplasia/CIN (with most of them, 133, being *down*-regulated, while 18 were seen *up*-regulated). Pathway analysis revealed enrichment of up-regulated genes in immune response-associated signalings (namely, innate antiviral defense), while down-regulated genes were expectedly enriched in epithelial differentiation pathways. Then samples were examined for their expression of invasion-associated genes (which included lympho-/angiogenesis, EMT, ECM disassembly, etc.), and 6 samples (3 CR vs. 3 CIN) were selected for further comparison. 201 DEGs identified were screened for immune-related genes, and a significant number of interferon-stimulated genes (OAS1-3, MX1, IRF9, ISG15, IFI44, etc.) were found to be up-regulated in a subset of microinvasive and early-stage invasive cancer compared with CIN2-3/CIS (*see heat*map). The members of various innate immune DNA/RNA-sensing pathways (including TLRs-, NLRs-, AIM2/inflammasome-, IFI16-, RLRs/MAVS-dependent, and others) and antiviral response also showed increased expression. Overexpression of proinflammatory chemokines (CXCL9, CXCL10, CX3CL1, etc.) in these samples points to the enhanced capacity to recruit activated T/B cells relative to CIS. Although no significant differences in the expression of immune checkpoints were detected between invasive cancer and precursor lesions, the development of immune suppression in early invasive cancer was evidenced by increased levels of IDO1, LGALS9, IL4R, but decreased levels of IL18, CD24 and many other components of T-cell activation/costimulation and antigen-presentation.

DEGs ' Pathway enrichment

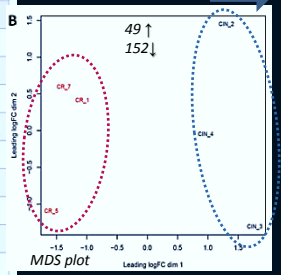
Direction	adj.Pval	nGenes	Pathways
Down regulated	1.0e-04	31	Cell death
	1.0e-04	14	Epidermis development
	1.0e-04	30	Programmed cell death
	6.1e-04	12	Skin development
	6.1e-04	7	Cornification
	7.1e-04	27	Tissue development
	8.4e-04	11	Epidermal cell differentiation
	9.8e-04	10	Keratinocyte differentiation
	1.6e-03	15	Epithelial cell differentiation
	6.2e-03	24	Apoptotic process
Up-regulated	8.2e-04	8	Immune response
	8.2e-04	7	Response to biotic stimulus
	8.2e-04	8	Interspecies interaction between organisms
	8.9e-04	3	Type I interferon signaling pathway
	1.0e-03	7	Defense response
	1.6e-03	8	Response to external stimulus
	3.5e-03	8	Immune system process
	5.6e-03	5	Innate immune response
	5.6e-03	3	Defense response to virus

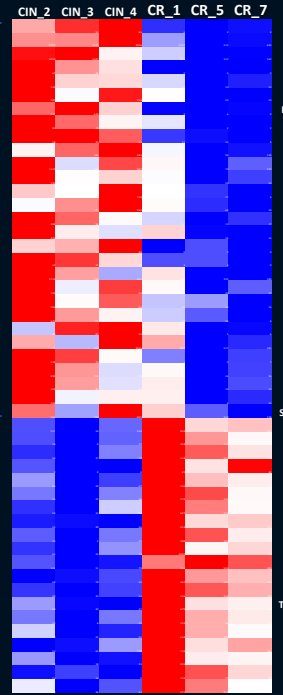
Number of DEGs (adj. Pval<0.05)

Comparisons	Up	Down
Norm-CR	145	184
Norm-CIN	0	0
CR-CIN	18	133

Angiogenesis, EMT, invasion

201DEGs





Pathway	Genes in List	Genes in Background	P-value	FDR	Genes
TLR	7	181	0,0009	0,0025	LAMP3, IL1RN, E2F7, CXCL10, GJB2, EREG, IL18
MAPK_only	12	559	0,0029	0,0025	ANPEP, OAS2, LM07, TNFRSF21, NT5E, SLC26A2, CD24, EDNRA, DKK1, EMP1, MTHFD1, KIAA1324
TNFa	8	259	0,0016	0,0028	TYMP, PPP1R3C, SLC7A2, GPR68, SLC39A8, PCDH18, MMP3, DKK1
IL-1	4	141	0,0321	0,0253	CX3CL1, UPP1, CXCL10, IL1RN
Wnt	3	83	0,0335	0,0258	HMGB2, CD24, DKK1

SPEED enrichment analysis revealed the **TLR-, TNF alpha-,** and **IL1**-dependent signalings among the top pathways lying behind the alterations of gene expression patterns observed at the initial stage of invasion.

pro-inflammatory
ISG
anti-inflammatory

Conclusion: The findings may contribute to a deeper understanding of the role of immune-relevant processes in cervical cancer expansion and dissemination, which is important for appropriate immunotherapy administration.

No conflicts of interest to declare

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