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ORIGINAL PAPERS

THE FALSE PREMISE OF THE PROSTATE CANCER GENE BIOMARKER PARADIGM

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Abstract. *Prostate tumor heterogeneity questions the value of the widely used gene biomarker paradigm for cancer diagnostic and therapy. We quantified the transcriptomic topologies and interplay of the toll-like receptors and chemokine signaling pathways in point biopsies of three cancer nodules and surrounding normal tissue from a surgically removed metastatic prostate. The analysis revealed that the cancer-related topology remodeling is different even between equally graded cancer nodules within the same tumor, pointing to distinct molecular mechanisms of the immune response. Our results invalidate the cancer biomarker paradigm and indicate the necessity to personalize gene anti-cancer therapy beyond individual patient to his/her cancer nodules' gene master regulators.*

Keywords: chemokine signaling, gene master regulators, genomic fabric paradigm, immune response, toll-like receptor signaling.

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Introduction

Prostate cancer (**PCa**), the out-of-control grow of the prostate gland, is the leading cancer-cause of death for men, with about 1 in 8 men being diagnosed during their lifetime. The incidence of PCa depends on age [1], race/ethnicity [2], diet and lifestyle [3], environment [4] and exposure to radiation and carcinogens [5].

There are several signs and symptoms used to diagnose both early and advanced stages of PCa but the most precise to date is considered the biopsy whose result can be graded using the Gleason score [6]. Clinicians and researchers are using a

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wide spectrum of molecular PCa biomarkers collected from blood [7], urine [8], sputum [9] or direct prostate biopsies.

As of October 5th, 2025, 50,366 PubMed listed publications reported investigations about biomarkers of prostate cancer (e.g. [10, 11], out of which, 10,441 publications were directly related to PCa gene biomarkers. *A PCa gene biomarker is a gene expressed in the prostate gland whose altered DNA sequence [12], 3D configuration [13] or expression level [14] is widely believed as always responsible for the cancerization of the tissue.* Conversely, restoration of the normal sequence, spatial configuration or normal expression of the biomarker was hoped to provide the cure.

The PCa gene biomarkers were selected by automated meta-analyses of large datasets of DNA sequences included in publicly accessible databases like TCGA - The Cancer Genome Atlas [15] and NCI – National (USA) Cancer Institute GDC Data Portal [16]. For gene expression alteration one may search the NCBI-GEO (Gene Expression Omnibus) the public functional genomic data repository of the National (USA) Center for Biotechnology Information [17]. During the years, IacobasLab from Albert Einstein College of Medicine (NY, 2001-2013), New York Medical College (2013-2017) or Prairie View A&M University (2017-) contributed with a total of 86 GEO datasets, out of which 18 were related to different forms of cancer [18]. Clinicians are also using commercial bioassays to detect various kinds of gene mutations [19, 20] or so-called "transcriptomic signatures" [21] of specific forms of cancer.

The large number of publications about cancer biomarkers is a direct consequence of the hundreds of millions of dollars invested in research and billions in revenues for pharma industry and hospitals. Yet we are still far from a definite therapeutic solution. Here, I will prove that the quasi-unanimously accepted Gene Biomarker Paradigm (**GBP**) in PCa research and clinical applications, is not only biologically false and misleading but also therapeutically irrelevant. For this, I will be using own GEO deposited genomic data obtained by profiling the gene expression from three cancer nodules and the surrounding normal tissue located in a surgically removed prostate tumor. The falsity of the GBP will be proven by showing that instead of having the same biomarker gene(s) significantly regulated in the cancerous prostates of all men, the regulomes are different even between equally pathologically graded cancerous nodules from the tumor of the same individual.

What makes GBP unreliable for PCa diagnostic and irrelevant for PCa therapy?

The blamed biomarker is not the single affected gene, the NCI GDC Data Portal listing mutation in 710 protein coding and 1 lncRNA genes responsible for 557 certified PCa cases. However, given the stochastic nature of the chemistry of

DNA replication, 1/1000 bases are randomly mutated, so practically there is no unmutated gene at any time in every cell. In addition, together with the biomarker, expression of hundreds other genes are significantly regulated in cancer in unrepeatable combinations from person to person. Hence, practically no specificity for both mutations and "transcriptomic signature". Moreover, the meta-analyses combined data collected by different labs, using different platforms and protocols and ignored the high (~30%) technical noise of the used genomic platforms. Nonetheless, the predictive values of the commercial cancer test kits [19 - 21] are disputable given that the number of possible combinatorial outcomes [22] substantially exceeds the number of living males on the Earth (sic!).

Beyond biological considerations, there are several methodological critical points of the GBP. First: the meta-analyses used to identify the biomarkers combined data collected by different labs, performed with various technologies and protocols and ignored the high (~30 - 40%) technical noise of the used genomic platforms. Second: the biomarkers were selected by comparing the average characteristics of a population of PCa patients with one of healthy-considered males, sometimes with the standard human reference genome [23] or pangenome [24]. Nevertheless, the most legitimate reference is the cancer-free tissue surrounding the cancer nodules as adopted by Iacobaslab [25-27] and other groups [28 - 31].

A very important observation is that the PCa biomarkers were selected from the most frequently altered genes among the men affected by this mortal disease. For instance, the five most frequently PCa altered genes listed by the NCI in the most recent data portal are: *IDH1* (76.80%), *TP53* (46.78%), *ATRX* (36.45%), *CIC* (21.44%), *FUBP1* (8.77%). If such a gene is among the most frequently altered, it means that it is one of the least protected by the cellular homeostatic mechanisms as it happens for the least important players in cell life cycle. Therefore, restoring its normal sequence or expression level would be of little consequence and so practically of no therapeutic value. Part of the above criticism was included in the Editorial of a recently published Reprint Book [32].

Materials and methods to test the Gene Biomarker Paradigm

Functional pathways

GBP was tested on the pathways constructed by the KEGG (Kyoto Encyclopedia Genes and Genomes) for toll-like receptor signaling [33], hereafter denoted as "**TLR**" and chemokine signaling [34], hereafter denoted "**CHR**". TLRs are membrane bound receptors that play critical roles in pattern recognition and host defenses [35]. Activation of TLR signaling pathways might fight cancer by recognizing dangerous damage-associated molecular patterns [36]. Inflammatory-

immune response of the prostate to cancerization implies calling leukocytes to the site of cell depreciation using chemokines, small chemoattractant proteins that provides directional indications for the cell trafficking. The chemokines bind to chemokine (G-protein coupled) receptors expressed in the immune cells. The analyzed pathways shared 33 genes, out of which we quantified 28: *AKT1/2/3, CCL3/3L3, CCL4L2, CCL5, CHUK, CXCL9/10/11, IKBKB/G, MAP2K1, MAPK1/10, NFKB1, NFKBIA, PIK3CA/B/D, PIK3R1/2/3, RAC1, RELA, STAT1/2.*

Prostate samples and gene expression data

The experiments described in this study were conducted according to the guidelines of the Declaration of Helsinki. At the time of the experiment (2016), the study was part of Dr. D.A. Iacobas' project approved by the Institutional Review Boards (IRB) of the New York Medical College's (NYMC) and Westchester Medical Center (WMC) Committees for Protection of Human Subjects. The approved IRB (L11,376 from 2 October 2015) granted access to frozen cancer specimens from the WMC Pathology Archives and depersonalized pathology reports, waiving the patient's informed consent.

The specimens were taken from three adenocarcinoma nodules, hereafter denoted as "A"," B", "C", and the surrounding normal tissue "N", all collected from the surgically removed prostate tumor of a 65y old black man. Nodule "A", located in the left posterior quadrant was received a total Gleason score 9/10 (4 + 5), while the other two nodules were equally graded 8/10 (4 + 4). Every point biopsy was split in four and total RNA was extracted from each quarter, purified, reversed transcribed, fluorescently labeled and hybridized with Agilent-026652 Whole Human Genome Microarray 4x44K v2 [37]. The hybridization protocol and the raw data can be retrieved from the public GEO repository [38].

Gene expression level and variation

According to our standard protocol [39], the expression level of a properly quantified gene "i" in all samples of a particular condition/region "c" were normalized to the median gene and averaged (AVE) over the four biological replicas of that region.

$$\forall i \,\&\, \forall c \;\rightarrow\; AVE_i^{(c)} \equiv \frac{1}{4}\sum_{k=1}^{4}\left(\frac{\propto_{i;k}^{(c)}}{\langle \alpha_{i;k}^{(c)} \rangle}\right) \;\; with\; a_i^{(c)} = \frac{1}{4}\sum_{k=1}^{4} \propto_{i;k}^{(c)} \tag{1}$$

where: $\frac{\propto_{i;k}^{(c)}}{\langle \alpha_{i;k}^{(c)} \rangle}$ is the net fluorescence (background subtracted foreground) of spot probing that gene in replica k (=1,2,3,4) of region c.

Moreover, for each gene, we determined also the Relative Expression Variation (REV) across the biological replicas:

$$REV_i^{(c)} \equiv \frac{\sigma_i^{(c)}}{2AVE_i^{(c)}}\left(\sqrt{\frac{r_i}{\chi^2(\beta;r_i)}}-\sqrt{\frac{r_i}{\chi^2(1-\beta;r_i)}}\right) \quad (2)$$

REV is the midinterval chi-square estimate of the coefficient of variation.

Gene expression regulation

Expression of a gene is considered significantly regulated in a cancer nodule with respect to the normal surrounding tissue of the tumor if it satisfies the composite criterion of the absolute expression ratio "|x|" exceeding the cut-off "CUT" and the less than 0.05 p-value of the heteroscedastic t- test of means' equality. "CUT" is computed for each gene to account for the combined effects of the technical noise of the used platform and the variability across the biological replicates:

$$\begin{cases}\left|x_i^{(cancer/normal)}\right| > CUT_i^{(cancer/normal)} \\ p_i^{(cancer/normal)} < 0.05\end{cases} \quad (3)$$

Where**:**

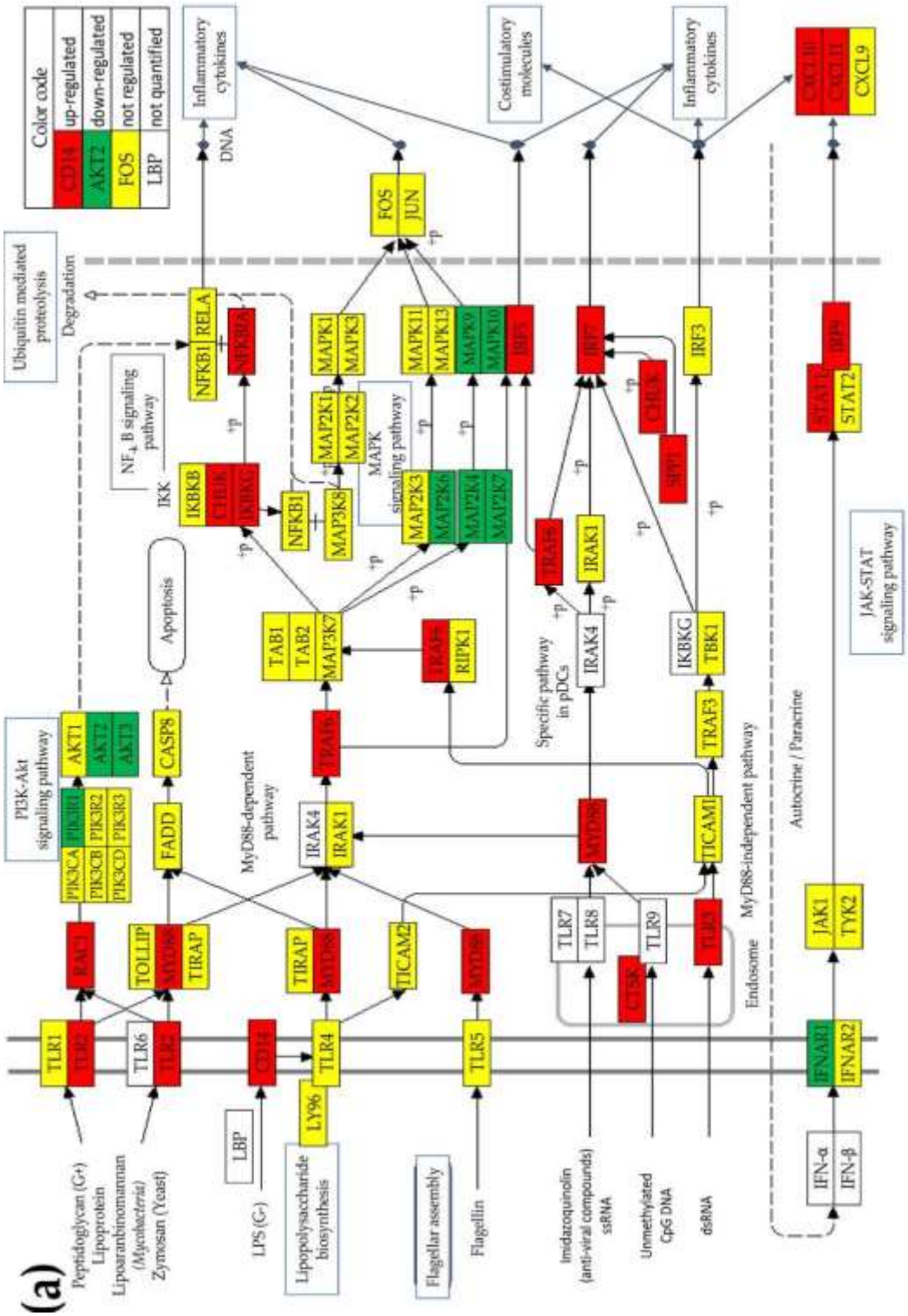
$$x_i^{(cancer/normal)} = \begin{cases}\dfrac{AVE_i^{(cancer)}}{AVE_i^{(normal)}}, & if\ AVE_i^{(cancer)} > AVE_i^{(normal)} \\ -\dfrac{AVE_i^{(normal)}}{AVE_i^{(cancer)}}, & if\ AVE_i^{(cancer)} \le AVE_i^{(normal)}\end{cases} \quad (4)$$

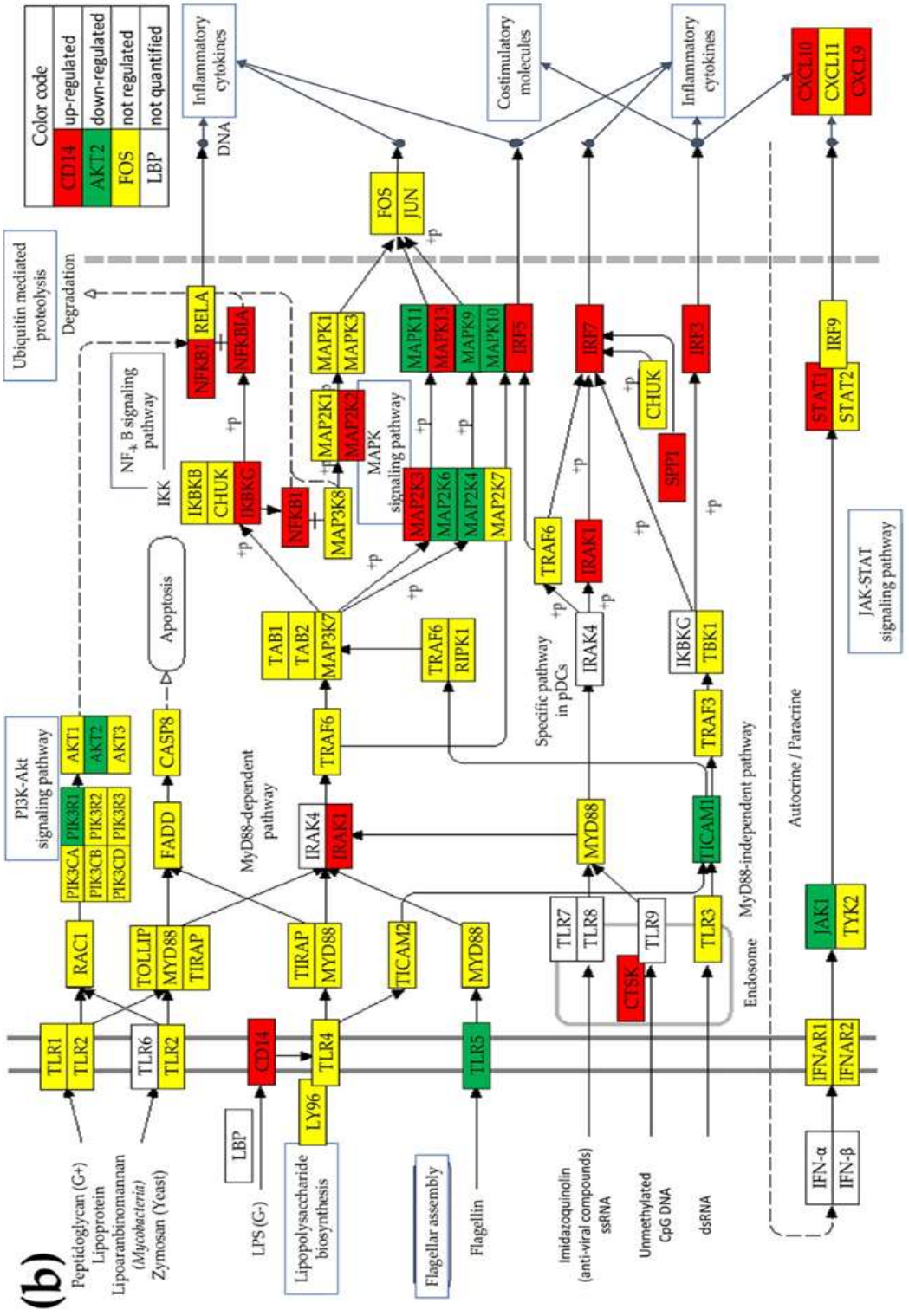
$$CUT_i^{(cancer/normal)} = 1+\sqrt{\frac{2}{100}\left(\left(REV_i^{(cancer)}\right)^2+\left(REV_i^{(normal)}\right)^2\right)} \quad (5)$$

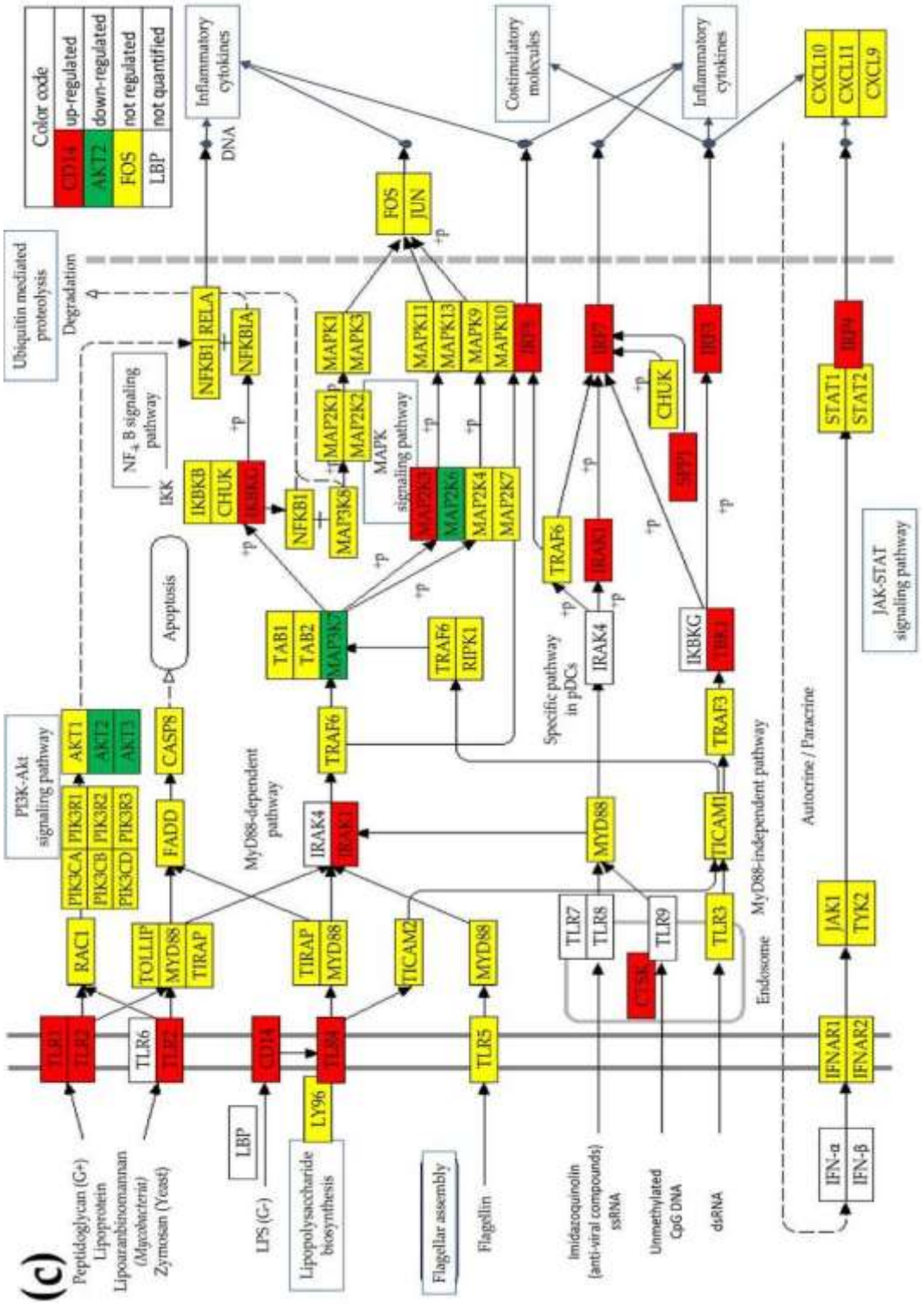
Results

Regulation of the toll-like receptors signaling (TLR) pathway

We profiled 76 out of 109 TLR KEGG-identified genes. Transcripts of missing genes were either not probed by the Agilent microarray platform, or their amount was below the detectable limit, or were hybridized to microarray spots with either corrupted or saturated pixels in at least one sample. Figure 1 presents the significantly regulated TLR signaling [33] genes in the cancer nodules "A", "B" and "C" with respect to "N".


Color code
up-regulated
down-regulated
not regulated
not quantified
CD14
AKT2
FOS
LBP
Inflammatory cytokines
Costimulatory molecules
Inflammatory cytokines
DNA
Ubiquitin mediated proteolysis
Degradation
FOS
JUN
NF-κB signaling pathway
IKK
MAPK signaling pathway
Apoptosis
PI3K-Akt signaling pathway
MyD88-dependent pathway
Specific pathway in pDCs
MyD88-independent pathway
Endosome
Autocrine / Paracrine
JAK-STAT signaling pathway
(a)
Peptidoglycan (G+)
Lipoprotein
Lipoarabinomannan (Mycobacteria)
Zymosan (Yeast)
LPS (G-)
Lipopolysaccharide biosynthesis
Flagellar assembly
Flagellin
Imidazoquinolin (anti-viral compounds) ssRNA
Unmethylated CpG DNA
dsRNA
IFN-α
IFN-β


(b)
Color code
CD14 up-regulated
AKT2 down-regulated
FOS not regulated
LBP not quantified
Inflammatory cytokines
Costimulatory molecules
Inflammatory cytokines
CXCL10 CXCL11 CXCL9
Ubiquitin mediated proteolysis
Degradation
FOS JUN
NF-κB signaling pathway
IKK
MAPK signaling pathway
JAK-STAT signaling pathway
PI3K-Akt signaling pathway
Apoptosis
MyD88-dependent pathway
Specific pathway in pDCs
MyD88-independent pathway
Autocrine / Paracrine
Endosome
Peptidoglycan (G+)
Lipoprotein
Lipoarabinomannan (Mycobacteria)
Zymosan (Yeast)
LPS (G-)
LBP
Lipopolysaccharide biosynthesis
Flagellar assembly
Flagellin
Imidazoquinolin (anti-viral compounds) ssRNA
Unmethylated CpG DNA
dsRNA
IFN-α
IFN-β
TLR1 TLR2
TLR6 TLR2
CD14
TLR4
TLR5
TLR7 TLR8
TLR9
TLR3
IFNAR1 IFNAR2
RAC1
TOLLIP MYD88 TIRAP
TIRAP MYD88
TICAM2
MYD88
CTSK
JAK1 TYK2
STAT1 STAT2 IRF9
PIK3CA PIK3R1
PIK3CB PIK3R2
PIK3CD PIK3R3
AKT1 AKT2 AKT3
FADD
CASP8
IRAK4 IRAK1
TRAF6
TAB1 TAB2 MAP3K7
TRAF6 RIPK1
NFKB1 RELA
NFKBIA
IKBKB CHUK IKBKG
NFKB1
MAP3K8
MAP2K1 MAP2K2
MAPK1 MAPK3
MAP2K3 MAP2K6 MAP2K4 MAP2K7
MAPK11 MAPK13 MAPK9 MAPK10
IRF5
TRAF6
IRAK4
MYD88
IRF7
IRAK1
CHUK
IKBKG TBK1
TRAF3
TICAM1
SPP1
IRF3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CXCL11
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Ubiquitin mediated proteolysis
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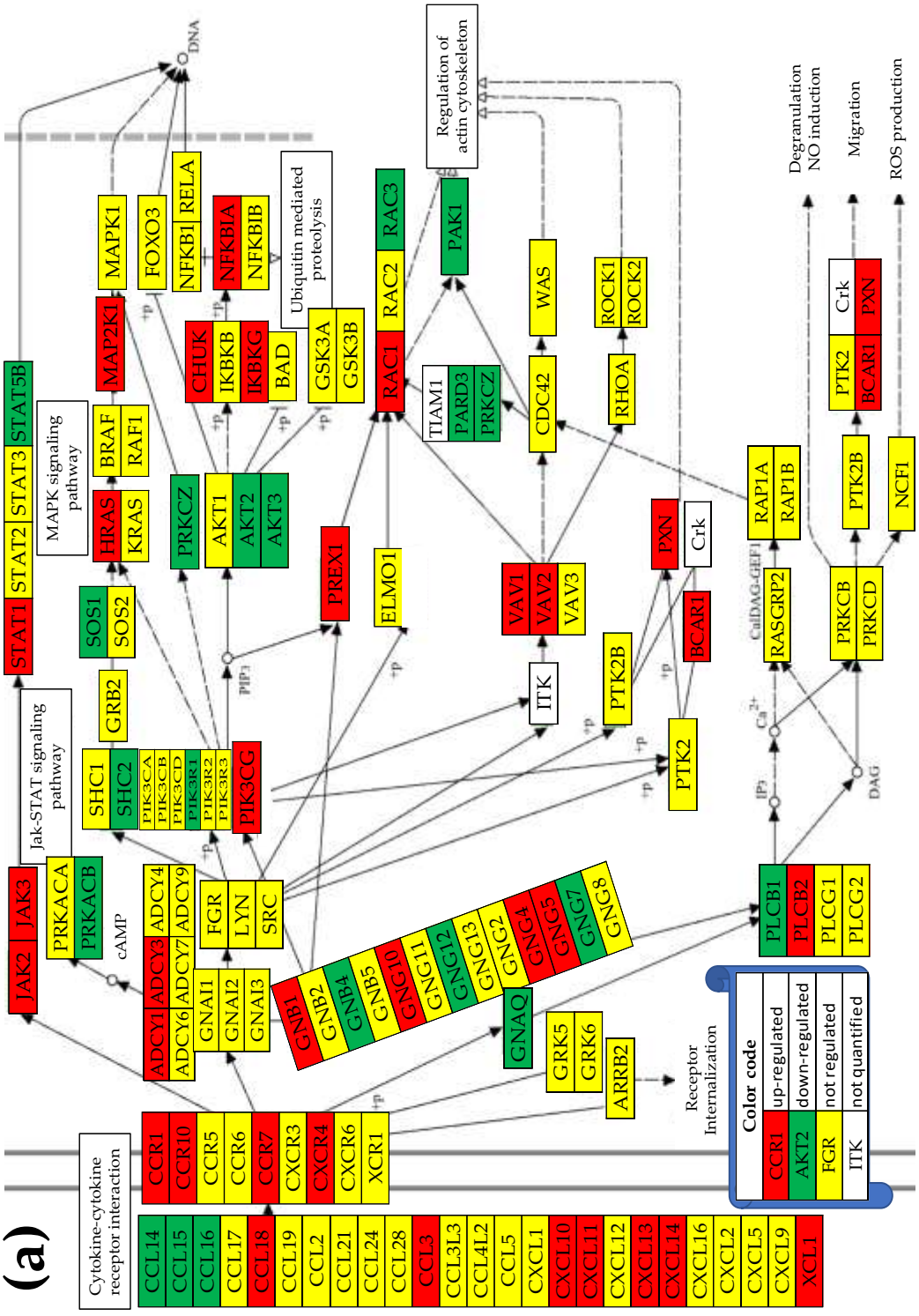
JUN
NF-κB signaling pathway
IKK
NFKB1
RELA
NFKBIA
MAPK1
MAPK3
MAPK11
MAPK13
MAPK9
MAPK10
MAPK
signaling pathway
MAP2K1
MAP2K2
IKBKB
CHUK
IKBKG
NFKB1
MAP3K8
MAP2K3
MAP2K6
MAP2K4
MAP2K7
IRF7
IRF3
CHUK
STAT1
STAT2
IRF9
JAK-STAT
signaling pathway
Apoptosis
TAB1
TAB2
MAP3K7
TRAF6
RIPK1
TRAF6
IRAK1
IRAK4
IKBKG
IRF3
TRAF3
PI3K-Akt
signaling pathway
AKT1
AKT2
AKT3
CASP8
PIK3CA
PIK3R1
PIK3CB
PIK3R2
PIK3CD
PIK3R3
FADD
MyD88-dependent
pathway
IRAK4
IRAK1
MYD88
TICAM1
Specific pathway
in pDCs
IRAK4
RAC1
TOLLIP
MYD88
TIRAP
TIRAP
MYD88
TICAM2
MYD88
TLR7
TLR8
TLR9
TLR3
JAK1
TYK2
Endosome
MyD88-independent pathway
Autocrine / Paracrine
TLR1
TLR2
TLR6
TLR2
CD14
TLR4
TLR5
IFNAR1
IFNAR2
LY96
LBP
IFN-α
IFN-β
(c)
Peptidoglycan (G+)
Lipoprotein
Lipoarabinomannan
(Mycobacterial)
Zymosan (Yeast)
LPS (G-)
Lipopolysaccharide
biosynthesis
Flagellar assembly
Flagellin
Imidazoquinolin
(anti-viral compounds)
ssRNA
Unmethylated
CpG DNA
dsRNA

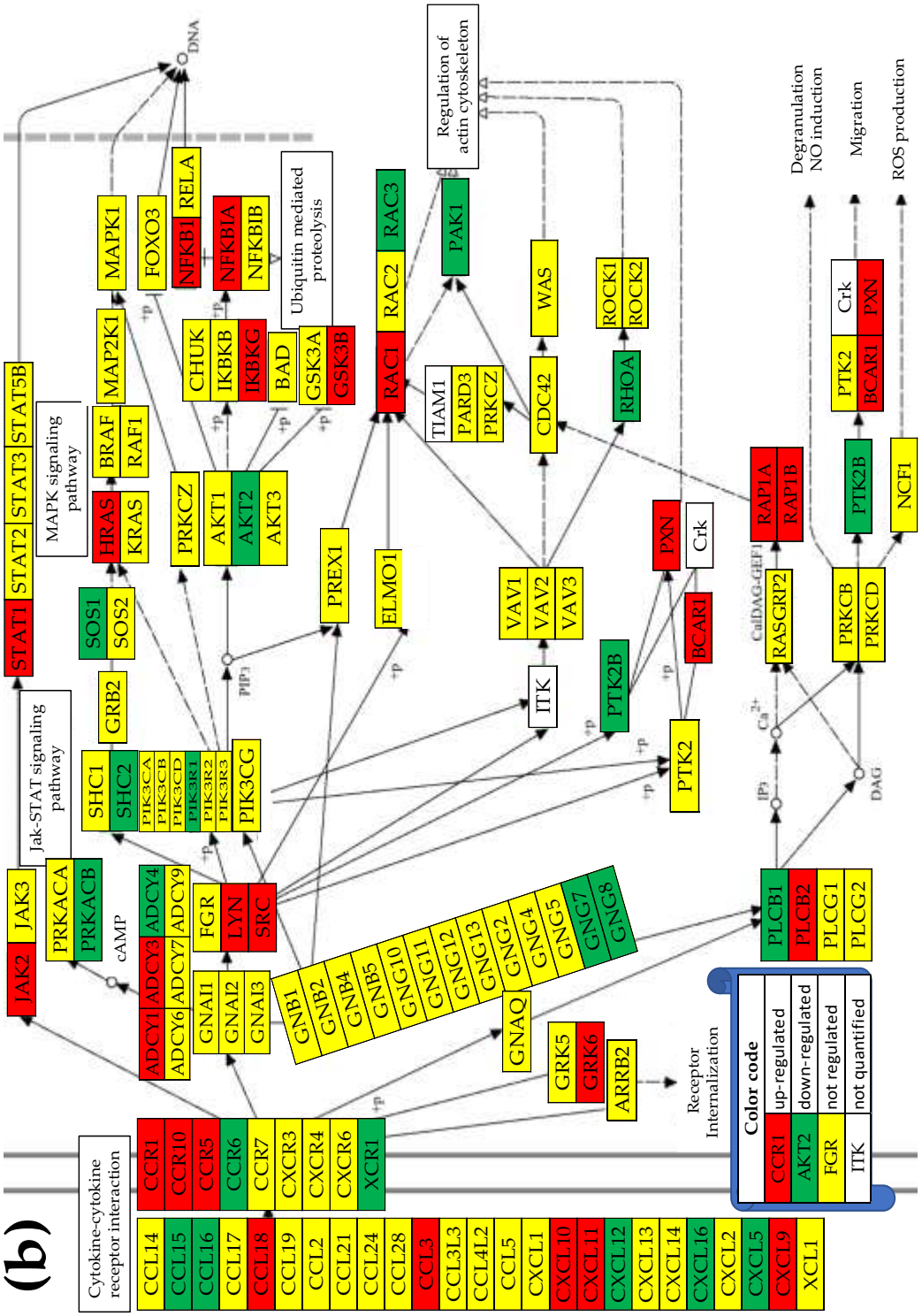
Figure 1: Significant expression regulation of the Toll-like receptors signaling pathway genes in cancer nodules "A" (a), "B" (b), and "C" (c) compared to **normal surrounding tissue "N".** Regulated genes: *AKT2/3* (v-akt murine thymoma viral oncogene homolog 2/3), *CCL3* (chemokine (C-C motif) ligand 3), *CD14* (CD14 molecule), *CHUK* (component of inhibitor of nuclear factor kappa B kinase complex), *CTSK* (cathepsin K), *CXCL9/10/11* (chemokine (C-X-C motif) ligand 9/10/11), *IFNAR1* (interferon alpha and beta receptor 1), *IKBKG* (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gam-ma), *IRAK1* (interleukin-1 receptor-associated kinase 1), *IRF3/5/7/9* (interferon regula-tory factor 3/5/7/9), *JAK1* (Janus kinase 1), *MAP2K1/2/3/4/6/7* (mitogen-activated pro-tein kinase kinase 1/2/3/4/6/7), *MAP3K7* (mitogen-activated protein kinase kinase kinase 7), *MAPK9/10/11/13* (mitogen-activated protein kinase 9/10/11/13), *MYD88* (myeloid differentiation primary response 88), *NFKB1* (nuclear factor of kappa light polypeptide gene enhancer in B-cells 1), *NFKBIA* (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha), *PIK3R1* (phosphoinositide-3-kinase, regulatory subunit 1 (alpha)), *RAC1* (ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)), *SPP1* (secreted phosphoprotein 1), *STAT1* (signal transducer and activator of transcription 1, 91kDa), *TBK1* (TANK-binding kinase 1), *TICAM1* (toll-like receptor adaptor molecule 1), *TLR1/2/3/4/5* (toll-like receptor 1/2/3/4/5), *TNF* (tumor necrosis factor), *TRAF6* (TNF receptor-associated factor 6, E3 ubiquitin protein ligase).

Of note is that even the equally graded nodules "B" and "C" exhibit differences in the gene regulation, proving their transcriptomic uniqueness. Thus, out of the 76 quantified genes, 17 (22.37%) were up- and 10 (13.16%) were down-regulated in "B", while 13 (17.11%) were up- and 4 (5.26%) were down-regulated in "C". Nodule "A" had 20 (26.32%) up- and 9 (11.84%) down-regulated genes. Although the three cancer nodules were differently regulated, no TLR gene was oppositely regulated in two nodules. Interesting, *CTSK*, a promoter of the cancer cell proliferation [40] and *CD14*, involved in tumor development [41], were upregulated in all cancer nodules.

Regulation of the chemokine signaling (CHS) pathway

Figure 2 presents the statistically significant regulation of the profiled 135 out of the 191 KEGG-identified CHS [34] genes in the cancer nodules "A", "B" and "C" with respect to the normal tissue "N". Of note is that even the equally graded nodules "B" and "C" exhibit differences in the gene regulation, proving the tumor transcriptomic heterogeneity. Thus, 24 (17.78%) were up- and 21 (15.56%) were down-regulated in "B", while 25 (18.52%) were up- and 8 (5.93%) were down-regulated in "C". Nodule "A" presented 32 (23.70%) up- and 20 (14.81%) down-regulated genes. Although the significant regulation profiles were different among the three cancer nodules, no CHS gene was oppositely regulated in two nodules.


(a)
Cytokine-cytokine receptor interaction
CCL14 CCL15 CCL16 CCL17 CCL18 CCL19 CCL2 CCL21 CCL24 CCL28 CCL3 CCL3L3 CCL4L2 CCL5 CXCL1 CXCL10 CXCL11 CXCL12 CXCL13 CXCL14 CXCL16 CXCL2 CXCL5 CXCL9 XCL1
CCR1 CCR10 CCR5 CCR6 CCR7 CXCR3 CXCR4 CXCR6 XCR1
Receptor Internalization
ARRB2 GRK5 GRK6
GNAQ GNB1 GNB2 GNB4 GNB5 GNG10 GNG11 GNG12 GNG13 GNG2 GNG4 GNG5 GNG7 GNG8
ADCY1 ADCY3 ADCY4 ADCY6 ADCY7 ADCY9 GNAI1 GNAI2 GNAI3
cAMP
PRKACA PRKACB
JAK2 JAK3
Jak-STAT signaling pathway
STAT1 STAT2 STAT3 STAT5B
FGR LYN SRC
SHC1 SHC2 GRB2 SOS1 SOS2
PIK3CA PIK3CB PIK3CD PIK3R1 PIK3R2 PIK3R3 PIK3CG
HRAS KRAS BRAF RAF1 MAP2K1 MAPK1
MAPK signaling pathway
FOXO3 NFKB1 RELA
CHUK IKBKB IKBKG BAD NFKBIA NFKBIB
Ubiquitin mediated proteolysis
GSK3A GSK3B
PRKCZ AKT1 AKT2 AKT3
PIP3
PREX1 ELMO1
RAC1 RAC2 RAC3 PAK1
Regulation of actin cytoskeleton
TIAM1 PARD3 PRKCZ
CDC42 WAS
RHOA ROCK1 ROCK2
VAV1 VAV2 VAV3
ITK
PTK2B
PXN Crk
BCAR1
PTK2
DNA
Color code
up-regulated
down-regulated
not regulated
not quantified
CCR1
AKT2
FGR
ITK
PLCB1 PLCB2 PLCG1 PLCG2
IP3
DAG
Ca^{2+}
CalDAG-GEF1
RASGRP2
RAP1A RAP1B
PRKCB PRKCD
PTK2B
NCF1
PTK2 Crk BCAR1 PXN
Degranulation
NO induction
Migration
ROS production


(b)
Cytokine-cytokine receptor interaction
CCL14 CCL15 CCL16 CCL17 CCL18 CCL19 CCL2 CCL21 CCL24 CCL28 CCL3 CCL3L3 CCL4L2 CCL5 CXCL1 CXCL10 CXCL11 CXCL12 CXCL13 CXCL14 CXCL16 CXCL2 CXCL5 CXCL9 XCL1
CCR1 CCR10 CCR5 CCR6 CCR7 CXCR3 CXCR4 CXCR6 XCR1
JAK2 JAK3
STAT1 STAT2 STAT3 STAT5B
Jak-STAT signaling pathway
PRKACA PRKACB
cAMP
ADCY1 ADCY3 ADCY4 ADCY6 ADCY7 ADCY9
GNAI1 GNAI2 GNAI3
GNB1 GNB2 GNB4 GNB5 GNG10 GNG11 GNG12 GNG13 GNG2 GNG4 GNG5 GNG7 GNG8
GNAQ
FGR LYN SRC
GRK5 GRK6
ARRB2
Receptor Internalization
SHC1 SHC2
GRB2
SOS1 SOS2
HRAS KRAS
BRAF RAF1
MAP2K1
MAPK1
MAPK signaling pathway
PIK3CA PIK3CB PIK3CD PIK3R1 PIK3R2 PIK3R3 PIK3CG
PRKCZ
AKT1 AKT2 AKT3
PIP3
FOXO3
NFKB1 RELA
NFKBIA NFKBIB
CHUK IKBKB IKBKG
BAD
GSK3A GSK3B
Ubiquitin mediated proteolysis
DNA
PREX1
ELMO1
RAC1 RAC2 RAC3
PAK1
TIAM1 PARD3 PRKCZ
Regulation of actin cytoskeleton
CDC42
WAS
RHOA
ROCK1 ROCK2
VAV1 VAV2 VAV3
ITK
PTK2B
PXN
Crk
BCAR1
PTK2
CalDAG-GEFI
RAP1A RAP1B
RASGRP2
PLCB1 PLCB2 PLCG1 PLCG2
IP3
DAG
PRKCB PRKCD
PTK2 Crk
BCAR1 PXN
PTK2B
NCF1
Degranulation
NO induction
Migration
ROS production
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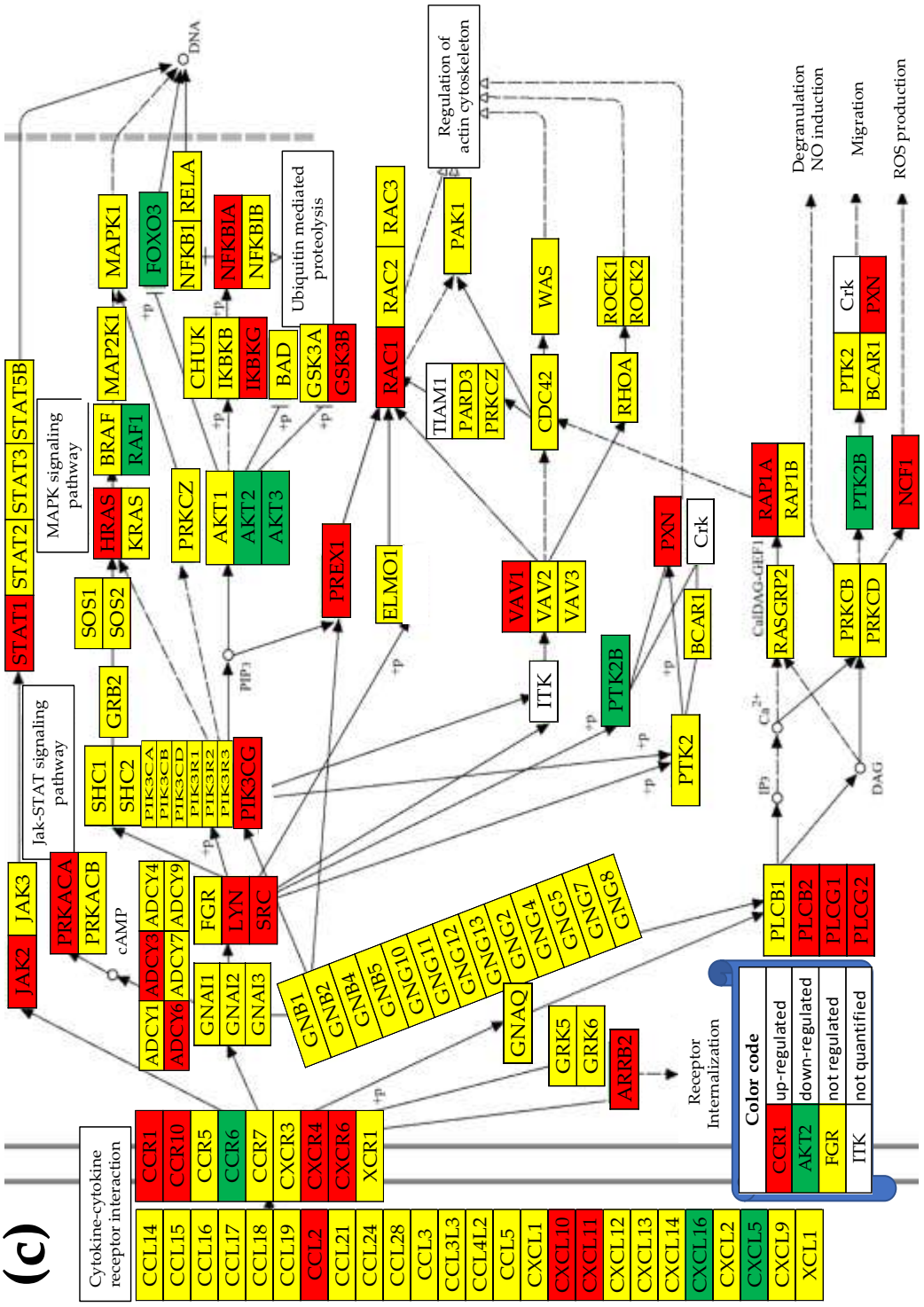
CCR1
AKT2
FGR
ITK


(c)
Cytokine-cytokine receptor interaction
CCL14
CCL15
CCL16
CCL17
CCL18
CCL19
CCL2
CCL21
CCL24
CCL28
CCL3
CCL3L3
CCL4L2
CCL5
CXCL1
CXCL10
CXCL11
CXCL12
CXCL13
CXCL14
CXCL16
CXCL2
CXCL5
CXCL9
XCL1
CCR1
CCR10
CCR5
CCR6
CCR7
CXCR3
CXCR4
CXCR6
XCR1
JAK2
JAK3
Jak-STAT signaling pathway
STAT1
STAT2
STAT3
STAT5B
cAMP
PRKACA
PRKACB
ADCY1
ADCY3
ADCY4
ADCY6
ADCY7
ADCY9
GNAI1
GNAI2
GNAI3
FGR
LYN
SRC
GNB1
GNB2
GNB4
GNB5
GNB10
GNB11
GNG12
GNG13
GNG2
GNG4
GNG5
GNG7
GNG8
GNAQ
GRK5
GRK6
ARRB2
Receptor Internalization
SHC1
SHC2
GRB2
SOS1
SOS2
HRAS
KRAS
BRAF
RAF1
MAP2K1
MAPK1
MAPK signaling pathway
PIK3CA
PIK3CB
PIK3CD
PIK3R1
PIK3R2
PIK3R3
PIK3CG
PRKCZ
AKT1
AKT2
AKT3
FOXO3
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IKBKB
IKBKG
NFKBIA
NFKBIB
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GSK3A
GSK3B
Ubiquitin mediated proteolysis
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ELMO1
RAC1
RAC2
RAC3
PAK1
TIAM1
PARD3
PRKCZ
Regulation of actin cytoskeleton
VAV1
VAV2
VAV3
ITK
CDC42
WAS
RHOA
ROCK1
ROCK2
PTK2B
PTK2
PXN
Crk
BCAR1
PRKCB
PRKCD
RAP1A
RAP1B
RASGRP2
CalDAG-GEFI
PLCB1
PLCB2
PLCG1
PLCG2
Ca2+
IP3
DAG
Degranulation
NO induction
Migration
ROS production
PTK2
BCAR1
Crk
PXN
NCF1
Color code
up-regulated
down-regulated
not regulated
not quantified
CCR1
AKT2
FGR
ITK

Figure 2: Significant expression regulation of chemokine signaling pathway genes in the cancer nodules "A" (a), "B" (b), and "C" (c) compared to normal tissue "N". Regulated genes: *ADCY1/3/4/6* (adenylate cyclase 1/3/4/6), *AKT2/3, ARBB1/2* (arrestin, beta 1/2), *BCAR1* (BCAR1 scaffold protein, Cas family member), *CCL2/3/14/15/16/18* (chemokine (C-C motif) ligand 2/3/14/15/16/18), *CHUK, CXCL5/9/10/11/12/13/14/16, FOXO3* (forkhead box O3), *GNAQ* (guanine nucleotide binding protein (G protein), q polypeptide), *GNB1/4* (guanine nucleotide binding protein (G protein), beta polypeptide 1/4), *GNG4/5/7/8/10/12* (guanine nucleotide binding protein (G protein), gamma 4,/5,/7,/8,/10,/and 12), *GRK6* (G protein-coupled receptor kinase 6), *GSK3B* (glycogen synthase kinase 3 beta), HRAS (Harvey rat sarcoma viral oncogene homolog), *IKBKG, JAK2/3* (Janus kinase 2/3), *LYN* (LYN proto-oncogene, Src family tyrosine kinase), *MAP2K1, MAPK10, NFKB1, PAK1* (p21 protein (Cdc42/Rac)-activated kinase 1), *PARD3* (par-3 family cell polarity regulator), *PIK3CG* (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma), *PIK3R1, PLCB1/2* (phospholipase C, beta 1/2), *PLCG1/2* (phospholipase C, gamma 1/2), *PREX1* (phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1), *PRKACA/B* (protein kinase, cAMP-dependent, catalytic, alpha/beta), *PRKCZ* (protein kinase C, zeta), *PTK2B* (protein tyrosine kinase 2 beta), *PXN* (paxillin), *RAC1/3, RAF1* (Raf-1 proto-oncogene, serine/threonine kinase), *RAP1A/B* (member of RAS oncogene family), *RHOA* (ras homolog family member A), *SHC2* (SHC adaptor protein 2), *SRC* (SRC proto-oncogene, non-receptor tyrosine kinase), *STAT1/5B, VAV1/2* (vav 1/2 guanine nucleotide exchange factor), *XCL1* (chemokine (C motif) ligand 1), *XCR1* (chemokine (C motif) receptor 1).

Discussion

The substantial tumor transcriptomic heterogeneity, even between equally graded nodules, as illustrated for gene expression regulation in Figures 1bc and 2bc, questions the validity of the gene biomarker paradigm for the prostate cancer. The differential significant regulation patterns observed in nodules "B" and "C" not only as affected genes but also as percentages (22.37% vs. 17.11% up-regulated and 13.16% vs. 5.26% down-regulated TLR genes, respectively) emphasize the importance of considering intra-tumoral heterogeneity in therapeutic strategies [42 – 45]. For instance, while three TLR signaling mitogen-activated kinases (*MAP2K2, MAP2K3, MAPK13*) were upregulated and five (*MAP2K4, MAP2K6, MAPK9, MAPK10, MAPK11*) were down-regulated in "B", only *MAP2K3* was upregulated and *MAP2K6* was down-regulated in "C" (Fig.1). The differential regulation of the mitogen-activated kinases in the two equally graded nodules indicates that participation of the MAPK signaling [46] to the "development, invasion, metastasis, and drug resistance" [47] of cancer cells is not uniform even across the same prostate. Also, while four CHS signaling guanine nucleotide-binding proteins (*GNB1, GNG10, GNG4, GNG5*) were up-regulated and three (*GNB4, GNG12, GNG7*) were down-regulated in "A", only two genes (*GNG7, GNG8*) were down-regulated in "B" and no gene was up- or down-regulated in "C" (Fig.2).

Conclusions

1 Although limited to one case of prostate cancer, the present study proved that beyond similarities, not only every man develops a unique form of prostate cancer but even within the same tumor each cancer nodule has its own "transcriptomic personality". The gene expression differences occurring even between equally pathologically graded cancer nodules in the same tumor makes unlikely the transcriptomic uniformity across distinct individuals as predicted by GBP. Despite here we investigated only two (albeit major) functional pathways, most likely, inter-nodule transcriptomic differences extend to all other components of the immune system.

2 GBP was invalidated also by our analysis of: mTOR signaling, metabolic pathways and expression coordination of *AKT2* with its partners central to the KEGG-constructed PCa pathway [48] in another prostate tumor [49]. Similar conclusion was obtained by us for the P53-signaling, apoptosis, block of differentiation, evading apoptosis, immortality, insensitivity to anti-growth signals, proliferation, resistance to chemotherapy, and sustained angiogenesis [50]. We found also that the immortalized PCa cell lines LNCaP and DU145 exhibited substantial differences in the gene expression compared to the standard prostate tissue [51, 52].

3 Altogether, the observed uniqueness of the gene expression profiles requires development of personalized gene therapy approach that has to go even to cancer nodule level, targeting the gene master regulators as proposed and tested by us on prostate, kidney and thyroid cancers [53 - 55].

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