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Prioritization of potential pharmacological targets for the development of anti-hepatocarcinoma drugs modulating the extrinsic apoptosis pathway: the reconstruction and analysis of associative gene networks help

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Abstract. Hepatocellular carcinoma (HCC) is a common severe type of liver cancer characterized by an extremely aggressive course and low survival rates. It is known that disruptions in the regulation of apoptosis activation are some of the key features inherent in most cancer cells, which determines the pharmacological induction of apoptosis as an important strategy for cancer therapy. The computer design of chemical compounds capable of specifically regulating the external signaling pathway of apoptosis induction represents a promising approach for creating new effective ways of therapy for liver cancer and other oncological diseases. However, at present, most of the studies are devoted to pharmacological effects on the internal (mitochondrial) apoptosis pathway. In contrast, the external pathway induced via cell death receptors remains out of focus. Aberrant gene methylation, along with hepatitis C virus (HCV) infection, are important risk factors for the development of hepatocellular carcinoma. The reconstruction of gene networks describing the molecular mechanisms of interaction of aberrantly methylated genes with key participants of the extrinsic apoptosis pathway and their regulation by HCV proteins can provide important information when searching for pharmacological targets. In the present study, 13 criteria were proposed for prioritizing potential pharmacological targets for developing anti-hepatocarcinoma drugs modulating the extrinsic apoptosis pathway. The criteria are based on indicators of the structural and functional organization of reconstructed gene networks of hepatocarcinoma, the extrinsic apoptosis pathway, and regulatory pathways of virus-extrinsic apoptosis pathway interaction and aberrant gene methylation-extrinsic apoptosis pathway interaction using ANDSystem. The list of the top 100 gene targets ranked according to the prioritization rating was statistically significantly (p-value = 0.0002) enriched for known pharmacological targets approved by the FDA, indicating the correctness of the prioritization method. Among the promising potential pharmacological targets, six highly ranked genes (*JUN*, *IL10*, *STAT3*, *MYC*, *TLR4*, and *KHDRBS1*) are likely to deserve close attention.
Key words: gene networks; hepatocarcinoma; programmed cell death; apoptosis; methylation.

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Приоритизация потенциальных фармакологических мишеней для создания лекарств против гепатокарциномы, модулирующих внешний путь апоптоза, на основе реконструкции и анализа ассоциативных генных сетей

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Аннотация. Гепатоцеллюлярная карцинома (ГЦК) – распространенный тяжелый тип рака печени, характеризующийся крайне агрессивным течением и низкой выживаемостью. Известно, что нарушения регуляции активации апоптоза являются одной из ключевых особенностей, свойственной большинству раковых клеток, что определяет фармакологическую индукцию апоптоза как важную стратегию терапии рака. Компьютерный дизайн химических соединений, способных целевым образом регулировать внешний сигнальный путь индукции апоптоза, представляет перспективный подход для создания новых эффективных средств терапии рака печени и других онкологических заболеваний. Однако в настоящее время большинство исследований посвящено фармакологическим воздействиям на внутренний (митохондриальный) путь апоптоза, тогда как внешний путь, индуцируемый посредством клеточных рецепторов смерти, остается вне поля зрения. Аберрантное метилирование генов наряду с инфекцией вирусом гепатита С считаются важными факторами риска развития ГЦК. Реконструкция генных сетей, описывающих молекулярные механизмы взаимодействия аберрантно метилированных генов с ключевыми участниками внешнего пути апоптоза, а также пути их регуляции белками вируса гепатита С, может дать важную информацию при поиске фармакологических мишеней. В настоящей работе были предложены 13 критериев приоритизации потенциальных фармакологических мишеней для создания лекарств против гепатокарциномы, модулирующих внешний путь апоптоза. В основу критериев легли показатели структурно-функциональной организации реконструированных с использованием ANDSystem генных сетей ГЦК, внешнего пути апоптоза и регуляторных путей взаимодействия «вирус – внешний путь апоптоза» и «аберрантное метилирование генов – внешний путь апоптоза». Список наиболее приоритетных 100 генов-мишеней, ранжированных согласно рейтингу приоритизации, оказался статистически значимо (p-value = 0.0002) обогащен известными фармакологическими мишенями, одобренными FDA, что указывает на корректность примененного метода приоритизации. Среди перспективных потенциальных фармакологических мишеней могут быть представлены шесть генов-кандидатов (*JUN*, *IL10*, *STAT3*, *MYC*, *TLR4* и *KHDRBS1*), занимающих высокое положение в ранжированном списке согласно результатам приоритизации.
Ключевые слова: генные сети; гепатокарцинома; программируемая клеточная гибель; апоптоз; метилирование.

Introduction

Hepatocellular carcinoma (HCC) is the most common tumor pathology of the liver, accounting for over 90 % of all malignant neoplasms of the liver and intrahepatic bile ducts (Llovet et al., 2018). It is characterized by an extremely aggressive course and low survival rate. Unlike most other types of cancer, there are some documented risk factors for the occurrence of HCC, such as infections caused by hepatitis C and B viruses, alcohol, fatty infiltration of the liver, hepatitis, autoimmune or chronic cholestatic diseases (Forner et al., 2012). Studies in the field of hepatocarcinogenesis have shown the critical role of genetic and epigenetic mechanisms leading to the formation of monoclonal populations of aberrant and dysplastic hepatocytes, which exhibit telomere erosion and re-expression of telomerase, microsatellite instability, and irreversible structural changes in genes and chromosomes (Balogh et al., 2016). The phenotype of malignant hepatocytes may be caused by the disruption of a number of genes that function in various regulatory pathways, resulting in different molecular variants of HCC (Thorgeirsson, Grisham, 2002). This characteristic of the pathology makes the reconstruction and analysis of gene networks describing the molecular mechanisms of the disease relevant.

In cancer therapeutic research, a central issue is suppressing cellular proliferation and the induction of programmed cell death. Apoptosis, one of the known mechanisms of programmed cell death, is divided into intrinsic and extrinsic, depending on the pathway of signal induction. The apoptosis signal induced by cell death receptors is called the extrinsic pathway, and the one induced by mitochondria – the intrinsic pathway (Krammer et al., 2007). In both cases, the apoptosis signal initiates the activation of caspases, key enzymes of apoptosis, leading to cell destruction, but the molecular mechanisms of signal transmission are entirely different. The literature focuses on regulating the intrinsic pathway of apoptosis, in which there has been certain progress in finding compounds with pharmacological potential for HCC therapy. It should be noted that the pharmacological effect on the extrinsic apoptosis pathway in HCC remains poorly studied. However, pharmacological induction of this pathway may bring significant, fundamentally important progress for cancer therapy.

Apoptosis induction is controlled by a range of inhibitor proteins, including c-FLIP, which blocks the activation of caspase-8, members of the anti-apoptotic BCl-2 family that inhibit the release of cytochrome C from mitochondria, and XIAP proteins that block the activation of caspase-3, -7, and -9. In the extrinsic apoptosis pathway, DISC, comprising PC, FADD, procaspase-8, -10 proteins, and c-FLIP, serves as a central platform for procaspase-8 activation (Lavrik, Krammer, 2012). c-FLIP can function within the DISC complex both pro- and anti-apoptotically. It is suggested that the formation of procaspase-8/c-FLIP heterodimers mediates the pro-apoptotic function of c-FLIP. Previously, in joint research conducted by the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences and the University of Magdeburg, we developed the world's first chemical probe (small chemical compound) capable of specifically binding to c-FLIP in the caspase-8/c-FLIP heterodimeric complex (Hillert et al., 2020). This small molecule was obtained by computer design and possessed biological activity – the ability to increase caspase-8 activity (Hillert et al., 2020).

Hepatitis C virus (HCV) is extensively studied in the scientific literature as a significant risk factor for HCC (Axley et al., 2018). The role of HCV has been shown in the regulation

of apoptosis and aberrant gene methylation, closely associated with HCC (Zheng et al., 2019; Lee, Ou, 2021).

Gene networks are widely used to describe the molecular-genetic mechanisms of various processes. We previously developed the software and information system ANDSystem (Ivanisenko V.A. et al., 2015, 2019; Ivanisenko T.V. et al., 2020, 2022), designed for the reconstruction and analysis of associative gene networks based on automatic knowledge extraction from scientific publications and factographic databases. Through the reconstruction of gene networks performed using ANDSystem, a number of studies have been conducted, such as the analysis of interactions of Hepatitis C virus proteins with the human proteome (Saik et al., 2016), the relationship of HCV with aberrant methylation in HCC (Antropova et al., 2022), interpretation of results of metabolome analysis of SARS-Cov-2 patients (Ivanisenko V.A. et al., 2022), tasks of prioritizing candidate genes associated with lymphedema, major depressive disorder (Yankina et al., 2018; Saik et al., 2019), search for new potential targets for drug action (Saik et al., 2018a, b), and others.

Based on the reconstruction and analysis of HCC gene networks and the extrinsic apoptosis pathway, as well as regulatory pathways linking HCV proteins with aberrantly methylated genes in HCC and key participants in the extrinsic apoptosis pathway, criteria were proposed for prioritizing potential pharmacological targets against HCC. Enrichment analysis of the first 100 target genes, ordered by prioritization results, showed significant content (p-value = 0.0002) in the list of FDA-approved pharmacological target genes, demonstrating the effectiveness of the proposed prioritization criteria. We suggest that the mechanism of action of drugs targeted at these targets is the modulation of the extrinsic apoptosis pathway, taking into account aberrant gene methylation, which could be utilized in creating a new class of drugs for HCC therapy. As promising potential pharmacological targets, ranked in the top thirty, the following candidate genes can be highlighted: *JUN*, *IL10*, *STAT3*, *MYC*, *TLR4*, and *KHDRBS1*.

Materials and methods

The ANDSystem software and information tool. Gene network reconstruction was performed using the ANDSystem software and information tool, automatically extracting knowledge from scientific publications and factual databases using artificial intelligence methods (Ivanisenko V.A. et al., 2019). ANDSystem includes a knowledge base containing over 40 million facts about molecular-genetic interactions, including physical intermolecular interactions, gene expression regulation, activity regulation, stability, and protein transport. Work on the reconstruction and analysis of gene networks in ANDSystem is performed using the ANDVisio program. The Pathway Wizard function implemented in ANDVisio was used to reconstruct regulatory pathways, which perform search queries to the knowledge base based on a given template. A schematic description of the templates used to reconstruct regulatory pathways is provided in Supplementary Materials 1–4[1].

Patient- and tissue-specific gene expression and DNA methylation data. Patient-specific and tissue-specific data on gene expression and DNA methylation were used to reconstruct gene networks. Tissue-specific gene expression data was used to filter gene networks using built-in ANDSystem methods. Information on tissue-specific gene expression was represented in ANDSystem. Information on differential gene expression was taken from the GEO database (Barrett et al., 2013; https://www.ncbi.nlm.nih.gov/geo/). Experiments were selected for which results of hepatocarcinoma tissue samples obtained from patients with this disease were available. The statistical significance values of differential gene expression and differential methylation in hepatocarcinoma tumor tissue samples compared to control samples were calculated in the GEO2R software package (Barrett et al., 2013; https://www.ncbi.nlm.nih.gov/geo/geo2r/). Calculation parameters were selected by default.

FDA-approved pharmacological targets. Data on FDA-approved pharmacological targets were extracted from the Human Protein Atlas resource (Uhlén et al., 2015; https://www.proteinatlas.org/).

Potential pharmacological target prioritization method. The criteria presented in Table 1 were used to prioritize candidate genes for pharmacological targets. The resulting gene weight was assessed as the sum of the weights of all criteria.

Results and discussion

To prioritize potential pharmacological targets, we applied 13 criteria considering various characteristics of the structural and functional organization of liver cancer gene networks and programmed cell death, including patient- and tissue-specific data on DNA methylation. Each criterion was assigned a quantitative weight indicator. The sum of the indicators for all 13 criteria was calculated as the resulting characteristic. To rank the genes by priority, they were arranged in a list from higher to lower values of the total indicator. Thus, genes with higher priority as candidates for pharmacological targets were at the top of the list (i. e., they had a lower rank).

When calculating the weight indicators of genes by prioritization criteria, the reconstruction of the gene networks of hepatocellular carcinoma (HCC) and the extrinsic apoptosis pathway was carried out as described below.

Reconstruction of the human hepatocellular carcinoma gene network

The automated search for genes associated with HCC, conducted using the new version of ANDSystem (Ivanisenko V.A. et al., 2019), identified more than 5,100 genes. Subsequently, ANDSystem built-in methods were used to filter genes by tissue specificity, retaining only the genes expressed in the liver – 4,905 genes. A list of 1,211 differentially expressed genes (DEGs) was then used based on RNA-seq analysis from the study by Huang et al. (2011). These data were obtained from the tissues of ten patients with HBV-associated HCC. Healthy tissues from the same patients were used as controls.

Following this step, the intersection of the gene network was reconstructed with ANDSystem, and the list of differentially expressed genes was carried out using ANDVisio built-in functions. As a result of the intersection, the gene network retained 584 genes found by ANDSystem methods to be associated with hepatocellular carcinoma based on data from published

[1] Supplementary Materials 1–7 are available at:
https://vavilov.elpub.ru/jour/manager/files/Suppl_Demenkov_Engl_27_7.pdf

Table 1. Criteria developed for prioritizing candidate genes of pharmacological targets

No.	Criterion name	Value	Characteristic
1	Gene representation in the HCC gene network	score1 = 2	The gene or the protein it encodes is represented in the gene network
		score1 = 0	The gene or the protein it encodes is not represented in the gene network
2	Gene representation in the extrinsic apoptosis gene network	score2 = 2	The gene or the protein it encodes is represented in the gene network
		score2 = 0	The gene or the protein it encodes is not represented in the gene network
3	Aberrant methylation indicator	score3 = 3	The gene is hypomethylated in HCC (there is data on increased expression)
		score3 = –5	The gene is hypermethylated (there is data on decreased expression)
4	Centrality indicator of the gene in regulatory pathways describing the regulation of key genes of the extrinsic apoptosis pathway (*CFLAR*, *CASP8*, and *FADD*) by genes from the HCC gene network (see Supplementary Material 1)	score4 = 1+ln(Q1)	The gene is represented in the regulatory gene network. Q1 – the number of connections of the gene with other nodes (degree centrality indicator)
		score4 = 0	The gene is not represented in the regulatory gene network
5	Centrality indicator of the protein in regulatory pathways describing the regulation of key genes of the extrinsic apoptosis pathway (*CFLAR*, *CASP8*, and *FADD*) by genes from the HCC gene network (see Supplementary Material 1)	score5 = 1+ln(Q2)	The protein is represented in the regulatory gene network. Q2 – the number of connections of the protein with other nodes (degree centrality indicator)
		score5 = 0	The protein is not represented in the regulatory gene network
6	Centrality indicator of the gene in regulatory pathways describing the regulation of key genes of the extrinsic apoptosis pathway (*CFLAR*, *CASP8*, and *FADD*) by HCV proteins (see Supplementary Material 2)	score6 = 2+ln(Q3)	The gene is represented in the regulatory gene network. Q3 – the number of connections of the gene with other nodes (degree centrality indicator)
		score6 = 0	The gene is not represented in the regulatory gene network
7	Centrality indicator of the protein in regulatory pathways describing the regulation of key genes of the extrinsic apoptosis pathway (*CFLAR*, *CASP8*, and *FADD*) by HCV proteins (see Supplementary Material 2)	score7 = 2+ln(Q4)	The protein is represented in the regulatory gene network. Q4 – the number of connections of the protein with other nodes (degree centrality indicator)
		score7 = 0	The protein is not represented in the regulatory gene network
8	Centrality indicator of the gene in regulatory pathways (see Supplementary Material 3) describing the regulation of hypermethylated genes by HCV proteins	score8 = ln(Q5)	The gene is represented in the regulatory gene network. Q5 – the number of connections of the gene with other nodes (degree centrality indicator)
		score8 = 0	The gene is not represented in the regulatory gene network
9	Centrality indicator of the protein in regulatory pathways (see Supplementary Material 3) describing the regulation of hypermethylated genes by HCV proteins	score9 = ln(Q6)	The protein is represented in the regulatory gene network. Q6 – the number of connections of the protein with other nodes (degree centrality indicator)
		score9 = 0	The protein is not represented in the regulatory gene network
10	Centrality indicator of the gene in regulatory pathways (see Supplementary Material 3) describing the regulation of hypomethylated genes by HCV proteins	score10 = 1+ln(Q7)	The gene is represented in the regulatory gene network. Q7 – the number of connections of the gene with other nodes (degree centrality indicator)
		score10 = 0	The gene is not represented in the regulatory gene network
11	Centrality indicator of the protein in regulatory pathways (see Supplementary Material 3) describing the regulation of hypomethylated genes by HCV proteins	score11 = 1+ln(Q8)	The protein is represented in the regulatory gene network. Q8 – the number of connections of the protein with other nodes (degree centrality indicator)
		score11 = 0	The protein is not represented in the regulatory gene network
12	Centrality indicator of the gene in regulatory pathways describing the regulation of key genes of the extrinsic apoptosis pathway (*CFLAR*, *CASP8*, and *FADD*) by aberrantly methylated genes (see Supplementary Material 4)	score12 = 2+ln(Q9)	The gene is represented in the regulatory gene network. Q9 – the number of connections of the gene with other nodes (degree centrality indicator)
		score12 = 0	The gene is not represented in the regulatory gene network
13	Centrality indicator of the protein in regulatory pathways describing the regulation of key genes of the extrinsic apoptosis pathway (*CFLAR*, *CASP8*, and *FADD)* by aberrantly methylated genes (see Supplementary Material 4)	score13 = 2+ln(Q10)	The protein is represented in the regulatory gene network. Q10 – the number of connections of the protein with other nodes (degree centrality indicator)
		score13 = 0	The protein is not represented in the regulatory gene network

works and databases, which were also present in the list of differentially expressed genes of human hepatocellular carcinoma obtained from RNA-seq data in (Huang et al., 2011). A search was then conducted for proteins expressed from these genes and metabolites associated with these proteins through direct interactions (a 'catalyst' type association), and a network of interactions between all objects in the gene network (genes, proteins, and metabolites) was reconstructed. The gene network contained 584 genes, 580 proteins, 1,061 metabolites, and over 16,000 interactions at this stage.

The gene network was expanded in the second stage with patient- and tissue-specific DNA methylation data (Supplementary Material 5). This included 67 genes with differentially altered methylation (hyper- or hypomethylated genes) in patient tumors compared to control samples. After adding aberrantly methylated genes and their protein products and expanding the gene network with metabolites interacting with them, the final gene network contained 627 genes, 624 proteins, 1,105 metabolites, and 17,387 interactions.

Reconstruction of the extrinsic apoptosis pathway gene network

The gene network of the extrinsic apoptosis pathway was reconstructed considering GeneOntology and ANDSystem data (Supplementary Material 6). Initially, a list of genes involved in the extrinsic apoptotic signaling pathway was formed using a query to the GeneOntology database. The following keywords were used for the query: GO term "extrinsic apoptotic signaling pathway", organism "human". Based on this query, a list of 259 genes was obtained. This list was then uploaded into the ANDVisio program to construct a gene network. Using ANDSystem, the gene network was expanded with proteins expressed from the entered genes, as well as with metabolites associated with these genes. As a result, the gene network of the extrinsic apoptosis pathway contained 259 genes, 260 proteins, and 513 metabolites.

Gene prioritization results

A total of 1,345 genes were analyzed, including participants in the HCC and extrinsic apoptosis pathway gene networks and regulatory pathways. The results of applying prioritization criteria for the top 30 priority genes are presented in Table 2. Out of 1,345 genes, 137 were targets of FDA-approved drugs. The top 100 priority list included 19 genes targeted by FDA-approved drugs. Detailed information on the results of prioritization for the 100 highest priority genes, containing quantitative values for each of the criteria, is provided in Supplementary Material 7. Of these 19 target genes, 17 are characterized as cancer-related genes. According to the hypergeometric distribution, the probability of an event in which 17 or more out of 19 selected genes are associated with cancer is $p = 0.0002$. This analysis signifies that the top 100 priority genes in the table of potential targets are statistically significantly associated with cancer (significance level $p = 0.0002$).

The calculation of prioritization criteria indicators, based on the reconstruction of regulatory pathways (criteria 4–13), was conducted automatically using ANDSystem with the templates provided in Supplementary Materials 1–4. The reconstruction and analysis of regulatory pathways of hypermethylated genes by Hepatitis C viral proteins, the results of which were used in prioritization criteria 8–11, have been previously described by us (Antropova et al., 2022).

The *JUN* gene occupies the top rank in the table (see Table 2). It belongs to the group of drug target genes approved by the FDA and is also associated with cancer (cancer-related genes). Numerous literature reports discuss its role in various types of cancer. For instance, it has been shown that JUN affects the development of colon cancer (Nateri et al., 2005) and that activated JUN is predominantly expressed at the invasive front of breast cancer and is associated with proliferation and angiogenesis (Vleugel et al., 2006).

According to our results, this gene could regulate the extrinsic apoptosis pathway. The regulatory network we reconstructed, which describes the molecular pathways through which JUN could regulate the extrinsic apoptosis pathway markers CFLAR, CASP8, and FADD, is presented in Figure 1. The regulatory network is based on various conclusions from experimental studies. For example, it has been shown that *FASLG* expression depends on JUN – irradiation increased FASLG expression in GCK cells via the activation of the JNK/c-Jun signaling pathway (Dong et al., 2016). The *FASLG* gene encodes the TNFL6 protein, a cytokine that binds to the TNFRSF6/FAS receptor, transmitting an apoptosis signal to cells. In another study (Liu Z. et al., 2019), deletion of *FASLG* inhibited the expression of *CASP8*, demonstrating another possible way for JUN to influence apoptosis (via CASP8).

It should be noted that pharmacological targets approved by the FDA, which are not associated with cancer but may be related to apoptosis, also present a particular interest. Specifically, in our table, *TLR4* (ranked 14th) stands out among such genes. According to the FDA, the *TLR4* gene is associated with "age-related macular degeneration" disease. Disruption of apoptosis is a key pathological factor in this disease (Yi et al., 2012).

The regulatory network describing the molecular pathways through which TLR4 can regulate CFLAR, CASP8, and FADD is presented in Figure 2. For instance, one can observe the regulatory influence from TLR4 to *TNFAIP3*. It is reconstructed based on a published study, showing that TLR4 activates a signaling pathway leading to the activation of NF-κB transcription factor. NF-κB, in turn, induces the expression of *TNFAIP3*, as demonstrated in endothelial cells (Soni et al., 2018). TNFAIP3 increases the level of cleaved caspase-8, as confirmed by knockdown, while overexpression of *TNFAIP3* has the opposite effect (Liu K. et al., 2018). Similarly, TLR4 could enhance the expression of *Beclin-1* through NF-κB (Copetti et al., 2009), which induces caspase-8 cleavage, leading to autophagy and apoptosis (Song et al., 2014).

The *IL10* gene occupies the second rank in the table. It belongs to the group of genes not included in the list of FDA-approved pharmacological targets. However, their mechanisms of influence on the development of HCC are widely discussed in the literature. In 2020, a study (Qian et al., 2020) suggested that combining IL10 and PD-L1 inhibitors may form the basis for effective treatment. The regulatory network, describing the molecular pathways through which IL10 can regulate CFLAR, CASP8, and FADD, is presented in Figure 3.

Another group consists of genes for which the FDA does not indicate approved agents, yet the mechanism of action of

Table 2. Top 30 genes ranked by priority level

Rank	Gene	Full gene name	Presence of FDA-approved* agents	Total weight
1	*JUN*	Proto-oncogene c-Jun	CR**	37.4
2	*IL10*	Interleukin-10	–	30.9
3	*STAT3*	Signal transducer and activator of transcription 3	–	30.1
4	*CASP8*	Caspase-8	–	29.4
5	*TP53*	Cellular tumor antigen p53	–	28.7
6	*CFLAR*	CASP8 and FADD-like apoptosis regulator	–	28.3
7	*MYC*	Myc proto-oncogene protein	–	23.7
8	*NFKB1*	Nuclear factor NF-kappa-B p105 subunit	CR	23.2
9	*FADD*	FAS-associated death domain protein	–	23.0
10	*IL33*	Interleukin-33	–	23.0
11	*ELAVL1*	ELAV-like protein 1	–	22.9
12	*FASLG*	Tumor necrosis factor ligand superfamily member 6	–	22.8
13	*TERT*	Telomerase reverse transcriptase	–	22.5
14	*TLR4*	Toll-like receptor 4	AR***	22.4
15	*BECN1*	Beclin-1	–	22.3
16	*CLDN1*	Claudin-1	–	22.3
17	*PARP1*	Poly [ADP-ribose] polymerase 1	CR	22.3
18	*TNFRSF1A*	Tumor necrosis factor receptor superfamily member 1A	CR	21.8
19	*CDKN1A*	Cyclin-dependent kinase inhibitor 1	–	21.6
20	*SP1*	Transcription factor Sp1	–	21.1
21	*KHDRBS1*	KH domain-containing, RNA-binding, signal transduction-associated protein 1	–	20.6
22	*MCL1*	Induced myeloid leukemia cell differentiation protein	–	20.6
23	*CLDN7*	Claudin-7	–	20.3
24	*CTSD*	Cathepsin D	–	20.0
25	*FASN*	Fatty acid synthase	CR	19.1
26	*MYCN*	N-myc proto-oncogene protein	–	18.7
27	*DDIT3*	DNA damage-inducible transcript 3 protein	–	18.4
28	*TNFAIP3*	Tumor necrosis factor alpha-induced protein 3	–	18.1
29	*STAT1*	Signal transducer and activator of transcription 1	–	17.6
30	*NLRP3*	NACHT, LRR and PYD domains-containing protein 3	–	17.6

* FDA – Food and Drug Administration, the agency of the US Department of Health and Human Services responsible for the sanitary supervision of food products and medicines; ** CR – cancer-related genes; *** AR – genes related to the "age-related macular degeneration" disease.

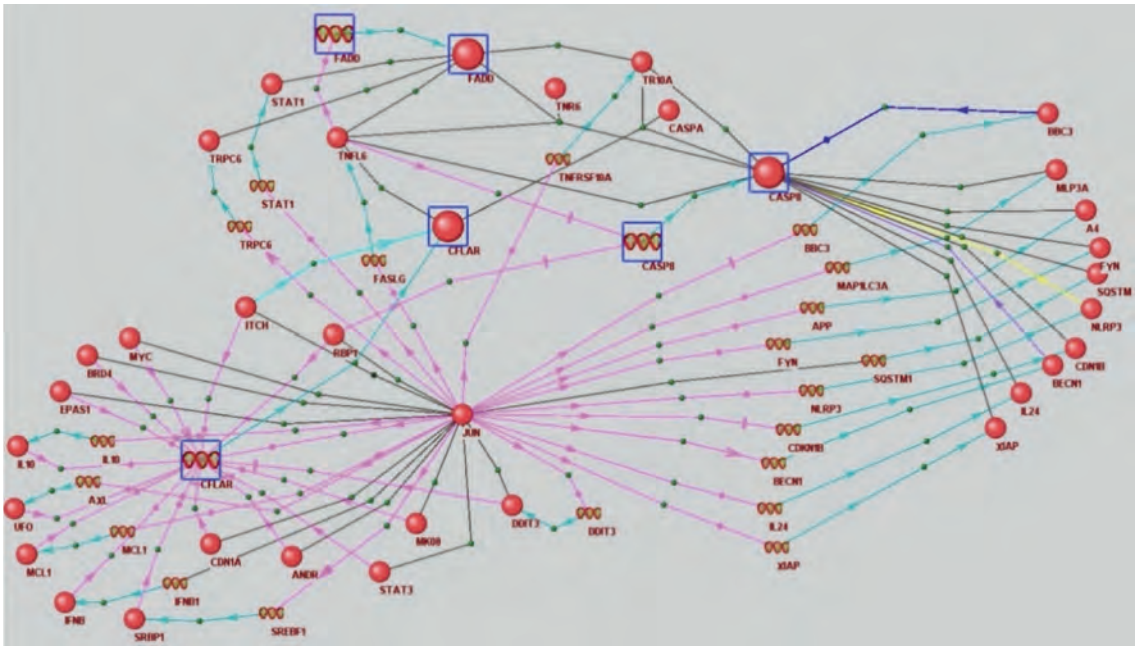

Fig. 1. Interaction network reconstructed using ANDSystem, through which JUN can regulate key apoptosis proteins – CFLAR, CASP8, and FADD.

Spheres represent proteins, and spirals symbolize genes. Black lines indicate physical interaction, turquoise arrows denote expression, pink arrows signify regulation of expression, blue arrows represent transport regulation, and yellow arrows indicate activity regulation.

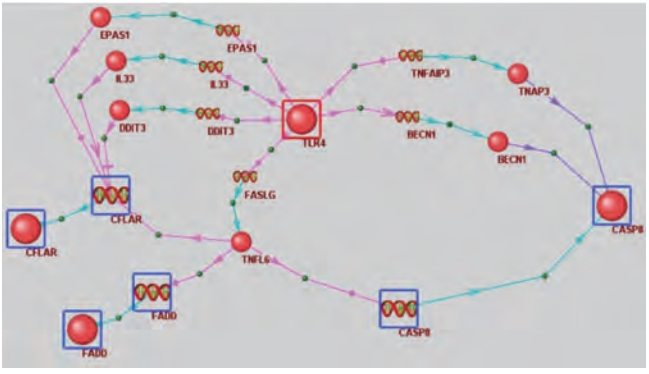

Fig. 2. Interaction network reconstructed using ANDSystem, through which TLR4 can regulate key apoptosis proteins – CFLAR, CASP8, and FADD.

Spheres represent proteins, and spirals symbolize genes. Turquoise arrows indicate expression, purple arrows represent regulation, and pink arrows denote expression regulation.

some widely used drugs affects these genes or the proteins they encode. This group includes the *STAT3* and *MYC* genes, occupying the rank table's third and seventh positions. A substantial number of publications indicate that STAT3 plays a crucial role in the initiation, progression, immune suppression, and metastasis of HCC. Specific drugs affect the functioning of STAT3. For instance, F.M. Gu et al. demonstrated that the inhibition of HCC growth and metastasis by the targeted anticancer drug "sorafenib" is mediated by blocking STAT3 (Gu et al., 2011). It is also known that sorafenib induces apoptosis (Xie et al., 2012). L. Wu et al., studying the mechanism of action of quercetin (a natural flavonoid included in some dietary supplements and drugs), showed that it inhibits the progression of HCC, affecting apoptosis, migration, invasion, autophagy, via the JAK2/STAT3 signaling pathway (at least partially) (Wu et al., 2019). The action mechanism of another anticancer drug – trametinib, used for melanoma treatment, is based on inhibiting the MEK protein, part of the signaling cascade. MEK inhibition reduces the MYC protein level, which promotes cell survival, and increases the pro-apoptotic protein BIM level, suppressing HCC growth (Zhou et al., 2019).

The direct markers of the extrinsic apoptosis pathway, *CASP8*, and *CFLAR*, are ranked 4th and 6th in the rank table. The *TP53* gene, the importance of which for apoptosis is well known, is positioned between them at the fifth position. Thus, it can be concluded that among the potential pharmacological targets we found, the top results of prioritization (see Table 2) include genes that are indeed drug targets – either FDA-approved or drugs aimed at other targets but affecting these genes and the proteins they encode in their action mechanisms, as well as genes that are only currently being discussed as promising targets.

Of particular interest as pharmacological targets may be genes that have been poorly studied to date in relation to HCC development mechanisms. Such genes could be fundamentally new pharmacological targets. Specifically, among such genes that made it to the top 100 highest priority list is *KHDRBS1*, which occupies the 21st position in the rank table (see Table 2). The regulatory network describing the molecular pathways through which KHDRBS1 can regulate CFLAR, CASP8, and FADD is presented in Figure 4.

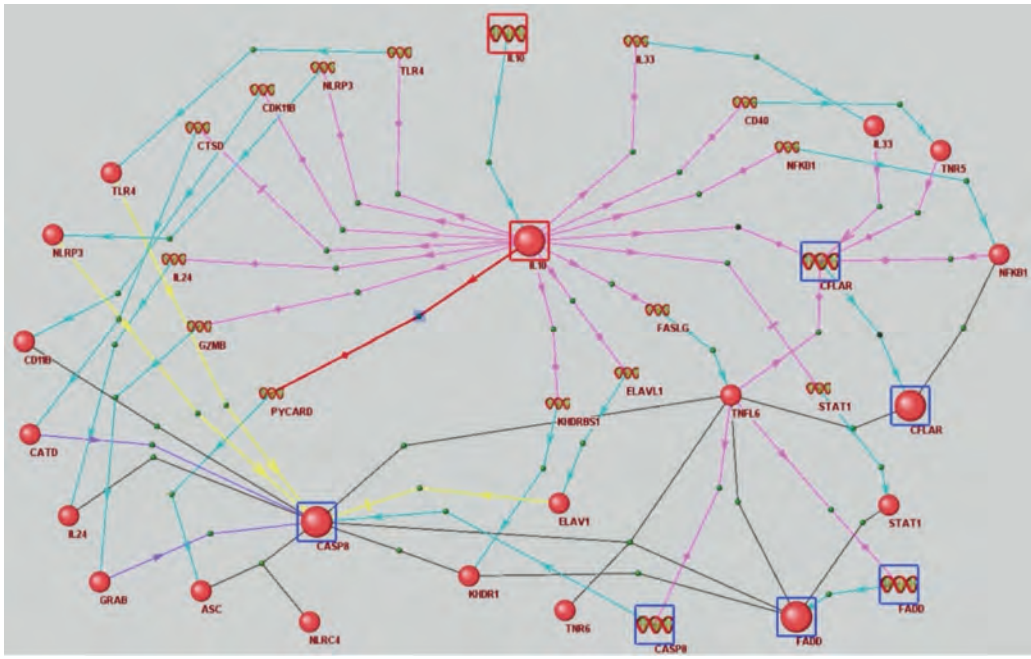

Fig. 3. Interaction network reconstructed using ANDSystem, through which IL10 can influence CFLAR, CASP8, and FADD.

Spheres represent proteins, and spirals symbolize genes. Black lines indicate physical interaction, turquoise arrows denote expression, purple arrows represent regulation, pink arrows signify regulation of expression, and yellow arrows indicate activity regulation.

Conclusion

A computer reconstruction of gene networks for hepatocellular carcinoma and programmed cell death (extrinsic apoptosis pathway) was conducted, taking into account patient- and tissue-specific DNA methylation data, using the ANDSystem software and information system. Based on the 13 developed criteria, considering the specifics of the reconstructed gene networks' structural and functional organization, potential pharmacological targets were prioritized. Six candidate genes (*JUN*, *IL10*, *STAT3*, *MYC*, *TLR4*, and *KHDRBS1*), occupying high positions in the ranked list according to prioritization results, may be of greatest interest as potential pharmacological targets.

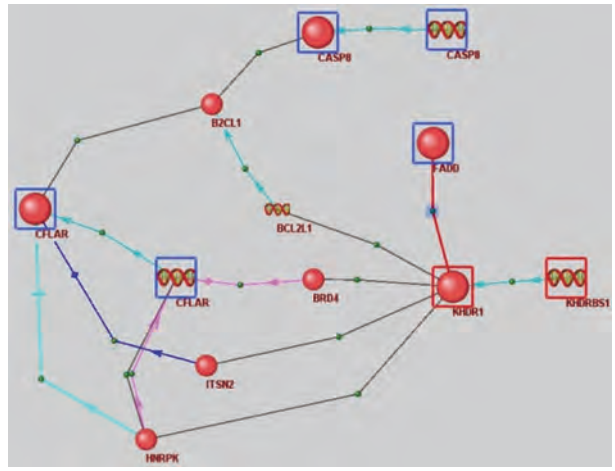

Fig. 4. Interaction network reconstructed using ANDSystem, through which KHDRBS1 can regulate key apoptosis proteins – CFLAR, CASP8, and FADD.

Spheres represent proteins, and spirals symbolize genes. Black lines indicate physical interaction, turquoise arrows denote expression, and pink arrows signify expression regulation.

References

Antropova E.A., Khlebodarova T.M., Demenkov P.S., Venzel A.S., Ivanisenko N.V., Gavrilenko A.D., Ivanisenko T.V., Adamovskaya A.V., Revva P.M., Lavrik I.N., Ivanisenko V.A. Computer analysis of regulation of hepatocarcinoma marker genes hypermethylated by HCV proteins. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding*. 2022;26(8):733-742. DOI 10.18699/VJGB-22-89

Axley P., Ahmed Z., Ravi S., Singal A.K. Hepatitis C virus and hepatocellular carcinoma: a narrative review. *J. Clin. Transl. Hepatol*. 2018;6(1):79-84. DOI 10.14218/JCTH.2017.00067

Balogh J., Victor D., Asham E.H., Burroughs S.G., Boktour M., Saharia A., Li X., Ghobrial R.M., Monsour H.P., Jr. Hepatocellular carcinoma: a review. *J. Hepatocell. Carcinoma*. 2016;3:41-53. DOI 10.2147/JHC.S61146

Barrett T., Wilhite S.E., Ledoux P., Evangelista C., Kim I.F., Tomashevsky M., Marshall K.A., Phillippy K.H., Sherman P.M., Holko M., Yefanov A., Lee H., Zhang N., Robertson C.L., Serova N., Davis S., Soboleva A. NCBI GEO: archive for functional genomics data sets – update. *Nucleic Acids Res*. 2013;41(D1):D991-D995. DOI 10.1093/nar/gks1193

Copetti T., Bertoli C., Dalla E., Demarchi F., Schneider C. p65/RelA modulates BECN1 transcription and autophagy. *Mol. Cell. Biol*. 2009;29(10):2594-2608. DOI 10.1128/MCB.01396-08

Dong Y., Shen X., He M., Wu Z., Zheng Q., Wang Y., Chen Y., Wu S., Cui J., Zeng Z. Activation of the JNK-c-Jun pathway in response to irradiation facilitates Fas ligand secretion in hepatoma cells and in-

creases hepatocyte injury. *J. Exp. Clin. Cancer Res*. 2016;35(1):114. DOI 10.1186/s13046-016-0394-z

Forner A., Llovet J.M., Bruix J. Hepatocellular carcinoma. *Lancet*. 2012;379(9822):1245-1255. DOI 10.1016/S0140-6736(11)61347-0

Gu F.M., Li Q.L., Gao Q., Jiang J.H., Huang X.Y., Pan J.F., Fan J., Zhou J. Sorafenib inhibits growth and metastasis of hepatocellular carcinoma by blocking STAT3. *World J. Gastroenterol*. 2011; 17(34):3922-3932. DOI 10.3748/wjg.v17.i34.3922

Hillert L.K., Ivanisenko N.V., Busse D., Espe J., König C., Peltek S.E., Kolchanov N.A., Ivanisenko V.A., Lavrik I.N. Dissecting DISC regulation via pharmacological targeting of caspase-8/c-FLIP$_L$ heterodimer. *Cell Death Differ*. 2020;27(7):2117-2130. DOI 10.1038/s41418-020-0489-0

Huang Q., Lin B., Liu H., Ma X., Mo F., Yu W., Li L., Li H., Tian T., Wu D., Shen F., Xing J., Chen Z.N. RNA-seq analyses generate comprehensive transcriptomic landscape and reveal complex transcript patterns in hepatocellular carcinoma. *PLoS One*. 2011;6(10):e26168. DOI 10.1371/journal.pone.0026168

Ivanisenko T.V., Saik O.V., Demenkov P.S., Ivanisenko N.V., Savostianov A.N., Ivanisenko V.A. ANDDigest: a new web-based module of ANDSystem for the search of knowledge in the scientific literature. *BMC Bioinformatics*. 2020;21(Suppl.11):228. DOI 10.1186/s12859-020-03557-8

Ivanisenko T.V., Demenkov P.S., Kolchanov N.A., Ivanisenko V.A. The new version of the ANDDigest tool with improved AI-based short names recognition. *Int. J. Mol. Sci.* 2022;23(23):14934. DOI 10.3390/ijms232314934

Ivanisenko V.A., Saik O.V., Ivanisenko N.V., Tiys E.S., Ivanisenko T.V., Demenkov P.S., Kolchanov N.A. ANDSystem: an Associative Network Discovery System for automated literature mining in the field of biology. *BMC Syst. Biol*. 2015;9(Suppl.2):S2. DOI 10.1186/ 1752-0509-9-S2-S2

Ivanisenko V.A., Demenkov P.S., Ivanisenko T.V., Mishchenko E.L., Saik O.V. A new version of the ANDSystem tool for automatic extraction of knowledge from scientific publications with expanded functionality for reconstruction of associative gene networks by considering tissue-specific gene expression. *BMC Bioinformatics*. 2019;20(Suppl.1):34. DOI 10.1186/s12859-018-2567-6

Ivanisenko V.A., Gaisler E.V., Basov N.V., Rogachev A.D., Cheresiz S.V., Ivanisenko T.V., Demenkov P.S., Mishchenko E.L., Khripko O.P., Khripko Y.I., Voevoda S.M. Plasma metabolomics and gene regulatory networks analysis reveal the role of nonstructural SARS-CoV-2 viral proteins in metabolic dysregulation in COVID-19 patients. *Sci. Rep*. 2022;12(1):19977. DOI 10.1038/s41598-022-24170-0

Krammer P.H., Kamiński M., Kiessling M., Gülow K. No life without death. *Adv. Cancer Res*. 2007;97:111-138. DOI 10.1016/S0065-230X(06)97005-5

Lavrik I.N., Krammer P.H. Regulation of CD95/Fas signaling at the DISC. *Cell Death Differ*. 2012;19(1):36-41. DOI 10.1038/cdd.2011.155

Lee J., Ou J.J. Hepatitis C virus and intracellular antiviral response. *Curr. Opin. Virol*. 2022;52:244-249. DOI 10.1016/j.coviro.2021.12.010

Liu K., Yao H., Wen Y., Zhao H., Zhou N., Lei S., Xiong L. Functional role of a long non-coding RNA LIFR-AS1/miR-29a/TNFAIP3 axis in colorectal cancer resistance to pohotodynamic therapy. *Biochim. Biophys. Acta Mol. Basis Dis*. 2018;1864(9B):2871-2880. DOI 10.1016/j.bbadis.2018.05.020

Liu Z., Fitzgerald M., Meisinger T., Batra R., Suh M., Greene H., Penrice A.J., Sun L., Baxter B.T., Xiong W. CD95-ligand contributes to abdominal aortic aneurysm progression by modulating inflammation. *Cardiovasc. Res*. 2019;115(4):807-818. DOI 10.1093/cvr/cvy264

Llovet J.M., Montal R., Sia D., Finn R.S. Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat. Rev. Clin. Oncol*. 2018;15(10):599-616. DOI 10.1038/s41571-018-0073-4

Nateri A.S., Spencer-Dene B., Behrens A. Interaction of phosphorylated c-Jun with TCF4 regulates intestinal cancer development. *Nature*. 2005;437(7056):281-285. DOI 10.1038/nature03914

Qian Q., Wu C., Chen J., Wang W. Relationship between IL10 and PD-L1 in liver hepatocellular carcinoma tissue and cell lines. *Biomed. Res. Int*. 2020;2020:8910183. DOI 10.1155/2020/8910183

Saik O.V., Ivanisenko T.V., Demenkov P.S., Ivanisenko V.A. Interactome of the hepatitis C virus: literature mining with ANDSystem. *Virus Res*. 2016;218:40-48. DOI 10.1016/j.virusres.2015.12.003

Saik O.V., Demenkov P.S., Ivanisenko T.V., Bragina E.Y., Freidin M.B., Dosenko V.E., Zolotareva O.I., Choynzonov E.L., Hofestaedt R., Ivanisenko V.A. Search for new candidate genes involved in the comorbidity of asthma and hypertension based on automatic analysis of scientific literature. *J. Integr. Bioinform*. 2018a;15(4):20180054. DOI 10.1515/jib-2018-0054

Saik O.V., Demenkov P.S., Ivanisenko T.V., Bragina E.Y., Freidin M.B., Goncharova I.A., Dosenko V.E., Zolotareva O.I., Hofestaedt R., Lavrik I.N., Rogaev E.I. Novel candidate genes important for asthma and hypertension comorbidity revealed from associative gene networks. *BMC Med. Genomics*. 2018b;11(1):61-76. DOI 10.1186/s12920-018-0331-4

Saik O.V., Nimaev V.V., Usmonov D.B., Demenkov P.S., Ivanisenko T.V., Lavrik I.N., Ivanisenko V.A. Prioritization of genes involved in endothelial cell apoptosis by their implication in lymphedema using an analysis of associative gene networks with ANDSystem. *BMC Med. Genomics*. 2019;12(Suppl.2):117-131. DOI 10.1186/s12920-019-0492-9

Song X., Kim S.Y., Zhang L., Tang D., Bartlett D.L., Kwon Y.T., Lee Y.J. Role of AMP-activated protein kinase in cross-talk between apoptosis and autophagy in human colon cancer. *Cell Death Dis*. 2014;5(10):e1504. DOI 10.1038/cddis.2014.463

Soni D., Wang D.M., Regmi S.C., Mittal M., Vogel S.M., Schlüter D., Tiruppathi C. Deubiquitinase function of A20 maintains and repairs endothelial barrier after lung vascular injury. *Cell Death Discov*. 2018;4:60. DOI 10.1038/s41420-018-0056-3

Thorgeirsson S.S., Grisham J.W. Molecular pathogenesis of human hepatocellular carcinoma. *Nat. Genet*. 2002;31(4):339-346. DOI 10.1038/ng0802-339

Uhlén M., Fagerberg L., Hallström B.M., Lindskog C., Oksvold P., Mardinoglu A., Sivertsson Å., Kampf C., Sjöstedt E., Asplund A., Olsson I., Edlund K., Lundberg E., Navani S., Szigyarto C.A., Odeberg J., Djureinovic D., Takanen J.O., Hober S., Alm T., Edqvist P.H., Berling H., Tegel H., Mulder J., Rockberg J., Nilsson P., Schwenk J.M., Hamsten M., von Feilitzen K., Forsberg M., Persson L., Johansson F., Zwahlen M., von Heijne G., Nielsen J., Pontén F. Proteomics. Tissue-based map of the human proteome. *Science*. 2015;347(6220):1260419. DOI 10.1126/science.1260419

Vleugel M.M., Greijer A.E., Bos R., van der Wall E., van Diest P.J. c-Jun activation is associated with proliferation and angiogenesis in invasive breast cancer. *Hum. Pathol*. 2006;37(6):668-674. DOI 10.1016/j.humpath.2006.01.022

Wu L., Li J., Liu T., Li S., Feng J., Yu Q., Zhang J., Chen J., Zhou Y., Ji J., Chen K., Mao Y., Wang F., Dai W., Fan X., Wu J., Guo C. Quercetin shows anti-tumor effect in hepatocellular carcinoma LM3 cells by abrogating JAK2/STAT3 signaling pathway. *Cancer Med*. 2019;8(10):4806-4820. DOI 10.1002/cam4.2388

Xie B., Wang D.H., Spechler S.J. Sorafenib for treatment of hepatocellular carcinoma: a systematic review. *Dig. Dis. Sci*. 2012;57(5):1122-1129. DOI 10.1007/s10620-012-2136-1

Yankina M.A., Saik O.V., Ivanisenko V.A., Demenkov P.S., Khusnutdinova E.K. Evaluation of prioritization methods of extrinsic apoptotic signaling pathway genes for retrieval of the new candidates associated with major depressive disorder. *Russ. J. Genet*. 2018; 54(11):1366-1374. DOI 10.1134/S1022795418110170

Yi H., Patel A.K., Sodhi C.P., Hackam D.J., Hackam A.S. Novel role for the innate immune receptor Toll-like receptor 4 (TLR4) in the regulation of the Wnt signaling pathway and photoreceptor apoptosis. *PLoS One*. 2012;7(5):e36560. DOI 10.1371/journal.pone.0036560

Zheng Y., Hlady R.A., Joyce B.T., Robertson K.D., He C., Nannini D.R., Kibbe W.A., Achenbach C.J., Murphy R.L., Roberts L.R., Hou L. DNA methylation of individual repetitive elements in hepatitis C virus infection-induced hepatocellular carcinoma. *Clin. Epigenetics*. 2019;11(1):145. DOI 10.1186/s13148-019-0733-y

Zhou X., Zhu A., Gu X., Xie G. Inhibition of MEK suppresses hepatocellular carcinoma growth through independent MYC and BIM regulation. *Cell. Oncol.* (*Dordr*.). 2019;42(3):369-380. DOI 10.1007/s13402-019-00432-4

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