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ORIGINAL RESEARCH ARTICLE

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# *E2F1* interactive with *BRCA1* pathway induces HCC two different small molecule metabolism or cell cycle regulation via mitochondrion or CD4+T to cytosol

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Breast cancer 1 (BRCA1) and E2F transcription factor 1 (E2F1) are related to metabolism and cell cycle regulation. However, the corresponding mechanism is not clear in HCC. High *BRCA1* direct pathway was constructed with 11 molecules from *E2F1* feedback-interactive network in HCC by GRNInfer based on 39 Pearson mutual positive corelation CC ≥0.25 molecules with *E2F1*. Integration of GRNInfer with GO, KEGG, BioCarta, GNF_U133A, UNIGENE_EST, Disease, GenMAPP databases by DAVID and MAS 3.0, *E2F1* feedback-interactive *BRCA1* indirect mitochondrion to cytosol pathway was identified as upstream *LAPTM4B* activation, feedback *UNG*, downstream *BCAT1-HIST1H2AD-TK1* reflecting protein, and DNA binding with enrichment of small molecule metabolism; The corresponding *BRCA1* indirect membrane to cytosol pathway as upstream *CCNB2-NUSAP1* activation, feedback *TTK-HIST1H2BJ-CENPF*, downstream *MCM4-TK1* reflecting ATP, and microtubule binding with enrichment of CD4+T-related cell cycle regulation in HCC. Therefore, we propose that *E2F1* interactive with *BRCA1* pathway induces HCC two different small molecule metabolism or cell cycle regulation via mitochondrion or CD4+T to cytosol. Knowledge analysis demonstrates our *E2F1* feedback-interactive *BRCA1* pathway wide disease distribution and reflects a novel common one of tumor and cancer.

**KEYWORDS**
CD4+T-related cell cycle regulation, from mitochondrion or membrane to cytosol, HCC, high *E2F1* feedback-interactive *BRCA1* pathway, small molecule metabolism

## 1 | INTRODUCTION

Based on DAVID (Huang et al., 2009a, 2009b) and MAS 3.0, breast cancer 1 (BRCA1) takes part in G2 DNA damage checkpoint, cellular protein metabolic process, positive regulation of cell cycle arrest, negative regulation of reactive oxygen species metabolic process, G2/M Checkpoint.

E2F transcription factor 1 (E2F1) takes part in DNA damage checkpoint, G1/S transition of mitotic cell cycle and regulation, negative regulation of transcription involved in G1/S transition of mitotic cell cycle, G1/S Check Point.

Qingchun Chen, Lin Wang, Minghu Jiang, and Juxiang Huang contributed equally to this work.

  

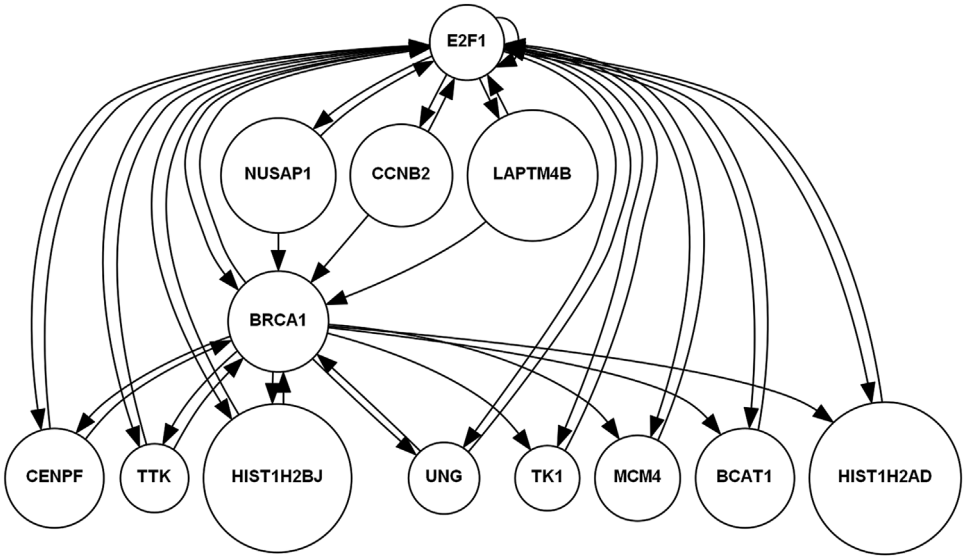


**FIGURE 1** *E2F1* feedback-interactive *BRCA1* direct complicated molecular pathway construction in HCC by GRNInfer based on Pearson mutual positive corelation CC ≥0.25 molecules with *E2F1*. Solid line with black arrow represents the activatory relationship and direction

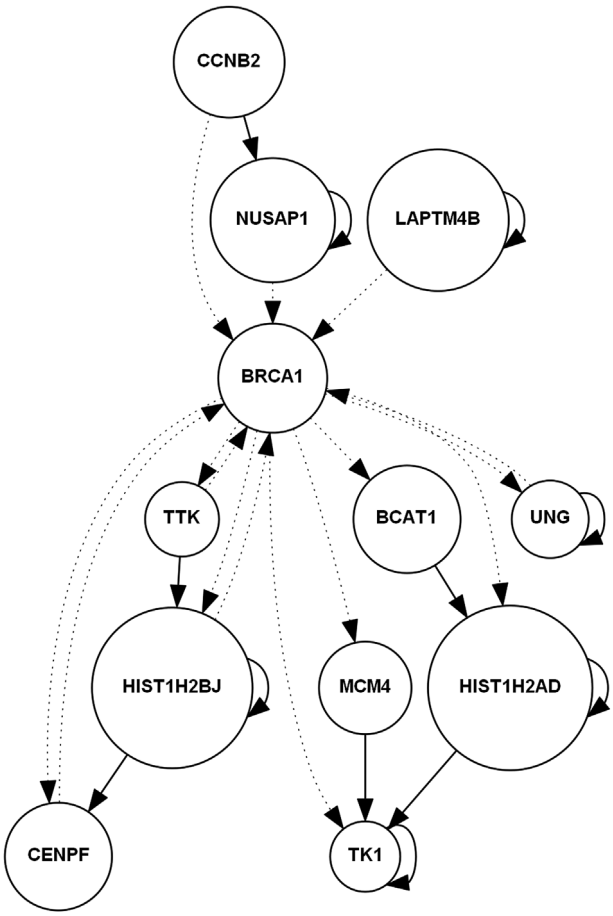


**FIGURE 2** *E2F1* feedback-interactive *BRCA1* complicated molecular pathway construction in HCC by GRNInfer based on Pearson mutual positive corelation CC ≥0.25 molecules with *E2F1*. Solid line with black arrow represents the indirect activatory relationship and direction with *BRCA1*, and dotted line with black arrow the direct activatory relationship and direction with *BRCA1*

*E2F1* has positive relationship with *BRCA1* reported in the reference. Such as, *E2F1*-mediated *BRCA1* expression by RNF126 contributes to promotion of homologous recombination (Shi, Wu, Ke, & Wang, 2015). Panobinostat suppressed the expression of *BRCA1* through downregulation of *E2F1* in acute myeloid leukemia (Xie et al., 2013). *KRT23* knockdown decreased the transcript and protein expression of *E2F1*, and *BRCA1* in colon cancer cell lines (Birkenkamp-Demtroder et al., 2013). *E2F1* was enriched at the *BRCA1* promoter in the demethylated UACC3199 cells (Xu et al., 2010). However, *E2F1* feedback-interactive *BRCA1* pathway-induced metabolism and cell cycle regulation mechanism has not been set forth from mitochondrion or membrane to cytosol in HCC.

The novel high *E2F1* feedback-interactive network was set up with 17 molecules by GRNInfer based on 39 Pearson mutual positive corelation CC ≥0.25 molecules with *E2F1* in HCC, although we have published some and established other single feedback/up/downstream activated, and inhibited direct and indirect molecular network based on our proposed knowledge hypotheses, and mechanisms (Diao et al., 2014; Huang et al., 2012a, 2012b; Huang, Wang, Jiang, & Lin, 2012; Qi et al., 2013; Wang et al., 2012a, 2012b, 2014; Wang, Huang, & Jiang, 2010a, 2010b; Wang, Huang, Jiang, & Lin, 2012; Wang, Huang, Jiang, & Sun, 2011a, 2011b).

In this paper, high *BRCA1* feedback/up/downstream direct and indirect activatory molecular pathway will be constructed from *E2F1* feedback-interactive network by GRNInfer, and the corresponding *BRCA1* indirect knowledge pathway based on GO, KEGG, BioCarta, GNF_U133A, UNIGENE_EST, Disease, GenMAPP databases by DAVID and MAS 3.0 in HCC. We will put forward and construct the new hypothesis, and mechanism of *E2F1* feedback-interactive *BRCA1* molecular pathway in HCC.

**TABLE 1** *BRCA1* indirect complicated feedback/up/downsream activatory pathway of biological process with occurrence numbers ≥2 from *E2F1* feedback-interactive network in HCC based on GO, KEGG, BioCarta, GNF_U133A, UNIGENE_EST, Disease, GenMAPP databases by DAVID and MAS 3.0

| **Biological process with occurrence numbers ≥2 of *E2F1* feedback-interactive *BRCA1* indirect pathway in HCC by DAVID and MAS 3.0** | | | | | |
|---|---|---|---|---|---|
| **Biological process** | **Num.** | **Molecules** | **Biological process** | **Num.** | **Molecules** |
| Upstream | | | | | |
| BM CD71 + early erythroid_3rd | 3 | CCNB2, LAPTM4B, NUSAP1 | Testis seminiferous tubule_3rd | 2 | CCNB2, LAPTM4B |
| Caudatenucleus_3rd | 3 | CCNB2, LAPTM4B, NUSAP1 | Testis Leydig cell_3rd | 2 | CCNB2, NUSAP1 |
| Cerebellum_3rd | 3 | CCNB2, LAPTM4B, NUSAP1 | Thalamus_3rd | 2 | CCNB2, LAPTM4B |
| Heart_3rd | 3 | CCNB2, LAPTM4B, NUSAP1 | Thyroid_3rd | 2 | CCNB2, NUSAP1 |
| Leukemia lymphoblastic (molt4)_3rd | 3 | CCNB2, LAPTM4B, NUSAP1 | Abdominal cavity_normal_3rd | 2 | CCNB2, LAPTM4B |
| Testis interstitial_3rd | 3 | CCNB2, LAPTM4B, NUSAP1 | Adrenal tumor_disease_3rd | 2 | CCNB2, LAPTM4B |
| Adipocyte_3rd | 2 | CCNB2, NUSAP1 | Cervical tumor_disease_3rd | 2 | CCNB2, LAPTM4B |
| BM CD33 + myeloid_3rd | 2 | CCNB2, NUSAP1 | Cervix_normal_3rd | 2 | CCNB2, LAPTM4B |
| Bronchial epithelial cells_3rd | 2 | CCNB2, NUSAP1 | Embryonic tissue_normal_3rd | 2 | CCNB2, LAPTM4B |
| CD4 + T cells_3rd | 2 | CCNB2, NUSAP1 | Gastrointestinal tumor_disease_3rd | 2 | CCNB2, LAPTM4B |
| Fetallung_3rd | 2 | CCNB2, NUSAP1 | Non glioma_disease_3rd | 2 | CCNB2, LAPTM4B |
| Leukemia promyelocytic (hl60)_3rd | 2 | CCNB2, NUSAP1 | Retinoblastoma_disease_3rd | 2 | CCNB2, LAPTM4B |
| Lymphnode_3rd | 2 | CCNB2, NUSAP1 | Pons_3rd | 2 | LAPTM4B, NUSAP1 |
| Lymphoma burkitts Raji_3rd | 2 | CCNB2, NUSAP1 | Germ cell tumor_disease_3rd | 2 | LAPTM4B, NUSAP1 |
| Testis germ cell_3rd | 2 | CCNB2, LAPTM4B | | | |
| Feedback | | | | | |
| Adipocyte_3rd | 4 | CENPF, HIST1H2BJ, TTK, UNG | Testis Leydig cell_3rd | 2 | CENPF, TTK |
| BM CD33 + myeloid_3rd | 4 | CENPF, HIST1H2BJ, TTK, UNG | Uterus_3rd | 2 | CENPF, HIST1H2BJ |
| BM CD71 + early erythroid_3rd | 4 | CENPF, HIST1H2BJ, TTK, UNG | Whole blood_3rd | 2 | CENPF, HIST1H2BJ |
| Leukemia lymphoblastic (molt4)_3rd | 4 | CENPF, HIST1H2BJ, TTK, UNG | Abdominal cavity_normal_3rd | 2 | CENPF, UNG |
| Lymphoma burkitts Raji_3rd | 4 | CENPF, HIST1H2BJ, TTK, UNG | Adrenal tumor_disease_3rd | 2 | CENPF, UNG |
| Germ cell tumor_disease_3rd | 4 | CENPF, HIST1H2BJ, TTK, UNG | Bladder_normal_3rd | 2 | CENPF, UNG |
| BM CD34 + _3rd | 3 | CENPF, HIST1H2BJ, TTK | Embryo_development_3rd | 2 | CENPF, HIST1H2BJ |
| Cerebellum_3rd | 3 | CENPF, HIST1H2BJ, TTK | Esophageal tumor_disease_3rd | 2 | CENPF, TTK |
| Heart_3rd | 3 | CENPF, TTK, UNG | Esophagus_normal_3rd | 2 | CENPF, TTK |
| Leukemia promyelocytic (hl60)_3rd | 3 | CENPF, TTK, UNG | Liver_normal_3rd | 2 | CENPF, HIST1H2BJ |
| Lymphnode_3rd | 3 | CENPF, TTK, UNG | Lymph node_normal_3rd | 2 | CENPF, TTK |

(Continues)

**TABLE 1** (Continued)

| Biological process with occurrence numbers ≥2 of *E2F1* feedback-interactive *BRCA1* indirect pathway in HCC by DAVID and MAS 3.0 | | | | | |
|---|---|---|---|---|---|
| **Biological process** | **Num.** | **Molecules** | **Biological process** | **Num.** | **Molecules** |
| Thalamus_3rd | 3 | CENPF, TTK, UNG | Mixed (normal and tumor) _disease_3rd | 2 | CENPF, TTK |
| Thyroid_3rd | 3 | CENPF, TTK, UNG | Small intestine_normal_3rd | 2 | CENPF, HIST1H2BJ |
| Colorectal tumor_disease_3rd | 3 | CENPF, TTK, UNG | Soft tissue/muscle tissue tumor_disease_3rd | 2 | CENPF, TTK |
| Embryonic tissue_normal_3rd | 3 | CENPF, TTK, UNG | Placenta_3rd | 2 | HIST1H2BJ, UNG |
| Breast (mammary gland) cancer_disease_3rd | 3 | HIST1H2BJ, TTK, UNG | Bone marrow_normal_3rd | 2 | HIST1H2BJ, TTK |
| Mitotic spindle assembly checkpoint | 2 | TTK, CENPF | Leukemia_disease_3rd | 2 | HIST1H2BJ, TTK |
| Regulation of cell cycle | 2 | TTK, CENPF | Placenta_normal_3rd | 2 | HIST1H2BJ, UNG |
| Amygdala_3rd | 2 | CENPF, UNG | Testis_normal_3rd | 2 | HIST1H2BJ, TTK |
| BM CD105 + endothelial_3rd | 2 | CENPF, HIST1H2BJ | Thymus_normal_3rd | 2 | HIST1H2BJ, TTK |
| CD4 + T cells_3rd | 2 | CENPF, TTK | Testis germ cell_3rd | 2 | TTK, UNG |
| Cingulate cortex_3rd | 2 | CENPF, HIST1H2BJ | Testis interstitial_3rd | 2 | TTK, UNG |
| Fetalbrain_3rd | 2 | CENPF, HIST1H2BJ | Infant (<3 years old) _development_3rd | 2 | TTK, UNG |
| Fetallung_3rd | 2 | CENPF, TTK | Juvenile (<17 years old) _development_3rd | 2 | TTK, UNG |
| PB CD19 + B cells_3rd | 2 | CENPF, HIST1H2BJ | Neonate (<4 weeks old) _development_3rd | 2 | TTK, UNG |
| Salivary gland_3rd | 2 | CENPF, HIST1H2BJ | Non glioma_disease_3rd | 2 | TTK, UNG |
| Smooth muscle_3rd | 2 | CENPF, HIST1H2BJ | Vascular_normal_3rd | 2 | TTK, UNG |
| Testis seminiferous tubule_3rd | 2 | CENPF, TTK | | | |
| Downstream | | | | | |
| Adipocyte_3rd | 4 | BCAT1, HIST1H2AD, MCM4, TK1 | BM CD105 + endothelial_3rd | 2 | BCAT1, HIST1H2AD |
| BM CD33 + myeloid_3rd | 4 | BCAT1, HIST1H2AD, MCM4, TK1 | Hypothalamus_3rd | 2 | BCAT1, HIST1H2AD |
| BM CD34 + _3rd | 4 | BCAT1, HIST1H2AD, MCM4, TK1 | Kidney_3rd | 2 | BCAT1, HIST1H2AD |
| BM CD71 + early erythroid_3rd | 4 | BCAT1, HIST1H2AD, MCM4, TK1 | Occipital lobe_3rd | 2 | BCAT1, HIST1H2AD |
| Cerebellum_3rd | 4 | BCAT1, HIST1H2AD, MCM4, TK1 | PB CD19 + B cells_3rd | 2 | BCAT1, HIST1H2AD |
| Leukemia lymphoblastic (molt4)_3rd | 4 | BCAT1, HIST1H2AD, MCM4, TK1 | Pons_3rd | 2 | BCAT1, HIST1H2AD |
| Lymphoma burkitts Raji_3rd | 4 | BCAT1, HIST1H2AD, MCM4, TK1 | Smooth muscle_3rd | 2 | BCAT1, HIST1H2AD |
| PB CD14 + monocytes_3rd | 4 | BCAT1, HIST1H2AD, MCM4, TK1 | Testis Leydig cell_3rd | 2 | BCAT1, MCM4 |
| Salivary gland_3rd | 4 | BCAT1, HIST1H2AD, MCM4, TK1 | Tonsil_3rd | 2 | BCAT1, HIST1H2AD |
| Superior cervical ganglion_3rd | 4 | BCAT1, HIST1H2AD, MCM4, TK1 | Whole Blood_3rd | 2 | BCAT1, HIST1H2AD |
| Lymph node_normal_3rd | 4 | BCAT1, HIST1H2AD, | Connective tissue_normal_3rd | 2 | BCAT1, |

(Continues)

**TABLE 1** (Continued)

| Biological process with occurrence numbers ≥2 of *E2F1* feedback-interactive *BRCA1* indirect pathway in HCC by DAVID and MAS 3.0 | | | | | |
|---|---|---|---|---|---|
| **Biological process** | **Num.** | **Molecules** | **Biological process** | **Num.** | **Molecules** |
| | | MCM4, TK1 | | | HIST1H2AD |
| Ovarian tumor_disease_3rd | 4 | BCAT1, HIST1H2AD, MCM4, TK1 | Embryo_development_3rd | 2 | BCAT1, HIST1H2AD |
| Ovary_normal_3rd | 4 | BCAT1, HIST1H2AD, MCM4, TK1 | Embryonic tissue_normal_3rd | 2 | BCAT1, MCM4 |
| Leukemia promyelocytic (hl60)_3rd | 3 | BCAT1, MCM4, TK1 | Uterine tumor_disease_3rd | 2 | BCAT1, HIST1H2AD |
| Placenta_3rd | 3 | BCAT1, HIST1H2AD, MCM4 | Temporal Lobe_3rd | 2 | HIST1H2AD, MCM4 |
| Germ cell tumor_disease_3rd | 3 | BCAT1, HIST1H2AD, MCM4 | Blood_normal_3rd | 2 | HIST1H2AD, TK1 |
| Leukemia_disease_3rd | 3 | BCAT1, MCM4, TK1 | Breast (mammary gland) cancer_disease_3rd | 2 | HIST1H2AD, TK1 |
| Salivary gland_normal_3rd | 3 | BCAT1, HIST1H2AD, MCM4 | Colon_normal_3rd | 2 | HIST1H2AD, TK1 |
| Testis_normal_3rd | 3 | BCAT1, HIST1H2AD, MCM4 | Colorectal tumor_disease_3rd | 2 | HIST1H2AD, TK1 |
| Bronchial epithelial cells_3rd | 3 | HIST1H2AD, MCM4, TK1 | Gastrointestinal tumor_disease_3rd | 2 | HIST1H2AD, TK1 |
| Thyroid_3rd | 3 | HIST1H2AD, MCM4, TK1 | Mammary gland_normal_3rd | 2 | HIST1H2AD, TK1 |
| Trachea_3rd | 3 | HIST1H2AD, MCM4, TK1 | Oral tumor_disease_3rd | 2 | HIST1H2AD, MCM4 |
| Cervical tumor_disease_3rd | 3 | HIST1H2AD, MCM4, TK1 | Thymus_normal_3rd | 2 | HIST1H2AD, MCM4 |
| Cervix_normal_3rd | 3 | HIST1H2AD, MCM4, TK1 | Tongue_normal_3rd | 2 | HIST1H2AD, MCM4 |
| Non glioma_disease_3rd | 3 | HIST1H2AD, MCM4, TK1 | Caudatenucleus_3rd | 2 | MCM4, TK1 |
| Skin tumor_disease_3rd | 3 | HIST1H2AD, MCM4, TK1 | CD4 + T cells_3rd | 2 | MCM4, TK1 |
| Skin_normal_3rd | 3 | HIST1H2AD, MCM4, TK1 | Fetallung_3rd | 2 | MCM4, TK1 |
| G1/S transition of mitotic cell cycle | 2 | MCM4, BCAT1 | Heart_3rd | 2 | MCM4, TK1 |
| Small molecule metabolic process | 2 | TK1, BCAT1 | Lymph node_3rd | 2 | MCM4, TK1 |
| Metabolic pathways | 2 | BCAT1, TK1 | Lymph_normal_3rd | 2 | MCM4, TK1 |

## 2 | METHODS

*BRCA1* complicated feedback/up/downstream activatory molecules and network were selected, and constructed from high *E2F1* feedback-interactive network in HCC by GRNInfer based on 225 significant expression molecules of 6,144 genes between 25 no-tumor hepatitis/cirrhotic tissues (HBV or HCV infection) and 25 HCC using GSE10140-10141 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE10140, http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE10141) by SAM (http://www-stat.stanford.edu/~tibs/SAM/) (The data processed by log base 2, two classes paired and minimum fold change ≥2 [the false-discovery rate 0%] selected). GRNInfer is one kind of Gene Network Reconstruction tool based on linear programming and a decomposition procedure (Wang, Joshi, Zhang, Xu, & Chen, 2006). The method for inferring gene networks is based on linear programming and decomposition procedure as the following equation (1).

$$J = (X' - A)U\Lambda^{-1}V^T + YV^T = \widehat{J} + YV^T \quad (1)$$

*BRCA1* complicated feedback/up/downstream activatory molecular pathway from high *E2F1* feedback-interactive network with only one direction was selected by deleting interaction with two opposite directions in HCC by programme.

**TABLE 2** *BRCA1* indirect complicated feedback/up/downsream activatory pathway of gene name, cellular component and molecular function with occurrence numbers from *E2F1* feedback-interactive network in HCC by DAVID

| Cellular component with occurrence numbers of *E2F1* feedback-interactive *BRCA1* indirect pathway in HCC by DAVID | | | | | |
|---|---|---|---|---|---|
| **GOTERM_CC_DIRECT** | **Num.** | **Molecules** | **GOTERM_CC_DIRECT** | **Num.** | **Molecules** |
| Upstream | | | | | |
| Membrane | 2 | LAPTM4B, CCNB2 | Integral component of membrane | 1 | LAPTM4B |
| Nucleoplasm | 1 | CCNB2 | Nucleus | 1 | NUSAP1 |
| Centrosome | 1 | CCNB2 | Chromosome | 1 | NUSAP1 |
| Cytosol | 1 | CCNB2 | Nucleolus | 1 | NUSAP1 |
| Microtubule cytoskeleton | 1 | CCNB2 | Cytoplasm | 1 | NUSAP1 |
| Endomembrane system | 1 | LAPTM4B | Spindle microtubule | 1 | NUSAP1 |
| Feedback | | | | | |
| Nucleus | 3 | HIST1H2BJ, UNG, CENPF | Cytosol | 1 | CENPF |
| Nucleoplasm | 3 | HIST1H2BJ, UNG, CENPF | Nuclear matrix | 1 | CENPF |
| Cytoplasm | 2 | HIST1H2BJ, CENPF | Midbody | 1 | CENPF |
| Spindle | 2 | TTK, CENPF | Pronucleus | 1 | CENPF |
| Chromosome | 1 | CENPF | Perinuclear region of cytoplasm | 1 | CENPF |
| Centromeric region | 1 | CENPF | Ciliary transition fiber | 1 | CENPF |
| Kinetochore | 1 | CENPF | Nucleosome | 1 | HIST1H2BJ |
| Chromatin | 1 | CENPF | Nuclear nucleosome | 1 | HIST1H2BJ |
| Spindle pole | 1 | CENPF | Extracellular space | 1 | HIST1H2BJ |
| Condensed chromosome outer kinetochore | 1 | CENPF | Membrane | 1 | TTK |
| Nuclear envelope | 1 | CENPF | Mitochondrion | 1 | UNG |
| Downstream | | | | | |
| Cytosol | 2 | TK1, BCAT1 | Nuclear chromosome | 1 | MCM4 |
| Nucleus | 2 | HIST1H2AD, MCM4 | Telomeric region | 1 | MCM4 |
| Mitochondrion | 1 | BCAT1 | Nucleoplasm | 1 | MCM4 |
| Nucleosome | 1 | HIST1H2AD | Membrane | 1 | MCM4 |
| Nuclear chromatin | 1 | HIST1H2AD | MCM complex | 1 | MCM4 |
| Extracellular exosome | 1 | HIST1H2AD | | | |
| Molecular function with occurrence numbers and gene name of E2F1 feedback-interactive BRCA1 indirect pathway in HCC by DAVID | | | | | |
| Upstream | | | | | |
| Protein binding | 3 | LAPTM4B, NUSAP1, CCNB2 | Cyclin B2 (CCNB2) | | |
| DNA binding | 1 | NUSAP1 | Lysosomal protein transmembrane 4 beta (LAPTM4B) | | |
| Microtubule binding | 1 | NUSAP1 | NUSAP1, nucleolar and spindle associated protein 1 (NUSAP1) | | |
| Poly(A) RNA binding | 1 | NUSAP1 | | | |
| Feedback | | | | | |
| Protein binding | 3 | TTK, UNG, CENPF | Protein serine/threonine/tyrosine kinase activity | 1 | TTK |
| Chromatin binding | 1 | CENPF | Protein tyrosine kinase activity | 1 | TTK |
| Protein C terminus binding | 1 | CENPF | ATP binding | 1 | TTK |
| Transcription factor binding | 1 | CENPF | Damaged DNA binding | 1 | UNG |

(Continues)

**TABLE 2** (Continued)

| Cellular component with occurrence numbers of *E2F1* feedback-interactive *BRCA1* indirect pathway in HCC by DAVID | | | | | |
|---|---|---|---|---|---|
| **GOTERM_CC_DIRECT** | **Num.** | **Molecules** | **GOTERM_CC_DIRECT** | **Num.** | **Molecules** |
| Protein homodimerization activity | 1 | CENPF | Uracil DNA N glycosylase activity | 1 | UNG |
| Dynein binding | 1 | CENPF | CENPF, centromere protein F (CENPF) | | |
| DNA binding | 1 | HIST1H2BJ | HIST1H2BJ, histone cluster 1, H2bj (HIST1H2BJ) | | |
| Protein heterodimerization activity | 1 | HIST1H2BJ | TTK, TTK protein kinase (TTK) | | |
| Protein serine/threonine kinase activity | 1 | TTK | UNG, uracil DNA glycosylase (UNG) | | |
| Downstream | | | | | |
| Identical protein binding | 2 | TK1, BCAT1 | Single stranded DNA binding | 1 | MCM4 |
| Protein binding | 2 | MCM4, TK1 | ATP dependent DNA helicase activity | 1 | MCM4 |
| ATP binding | 2 | MCM4, TK1 | Thymidine kinase activity | 1 | TK1 |
| Branched chain amino acid transaminase activity | 1 | BCAT1 | Zinc ion binding | 1 | TK1 |
| L leucine transaminase activity | 1 | BCAT1 | Nucleoside kinase activity | 1 | TK1 |
| L valine transaminase activity | 1 | BCAT1 | BCAT1, branched chain amino-acid transaminase 1, cytosolic (BCAT1) | | |
| L isoleucine transaminase activity | 1 | BCAT1 | HIST1H2AD, histone cluster 1, H2ad (HIST1H2AD) | | |
| DNA binding | 1 | HIST1H2AD | MCM4, minichromosome maintenance complex component 4 (MCM4) | | |
| Protein heterodimerization activity | 1 | HIST1H2AD | TK1, thymidine kinase 1, soluble (TK1) | | |

High *E2F1* feedback-interactive *BRCA1* direct molecular pathway was identified with relationship of *E2F1* and *BRCA1*, and the corresponding indirect complicated feedback/up/downstream activatory molecular pathway left without relationship of *E2F1* and *BRCA1* in HCC by programme, and drawn via GVedit tool.

Gene name, biological process, cellular component, molecular function, and occurrence numbers were constructed in *E2F1* feedback-interactive *BRCA1* indirect feedback/up/downstream pathway based on GO, KEGG, BioCarta, GNF_U133A, UNIGENE_EST, Disease, GenMAPP databases by DAVID (https://david.ncifcrf.gov/) and MAS 3.0 (CapitalBio Corporation, Beijing, China; http://bioinfo.capitalbio.com/mas3/) in HCC, respectively.

New knowledge hypothesis and mechanism were proposed based on *E2F1* feedback-interactive *BRCA1* indirect complicated knowledge pathway, and the corresponding *BRCA1* indirect molecular pathway identified in HCC on this basis.

## 3 | RESULTS

High *E2F1* feedback-interactive *BRCA1* direct molecular pathway was constructed from 11 molecules upstream *NUSAP1*, *CCNB2*, *LAPTM4B*; feedback *CENPF*, *TTK*, *HIST1H2BJ*, *UNG*; downstream *TK1*, *HIST1H2AD*, *MCM4*, *BCAT1* in HCC based on GRNInfer, as shown in Figure 1.

High *E2F1* feedback-interactive *BRCA1* indirect molecular pathway shows upstream *LAPTM4B*, *or CCNB2-NUSAP1 activation;* feedback *UNG*, *or TTK-HIST1H2BJ-CENPF*; downstream *MCM4 or BCAT1-HIST1H2AD to TK1* in HCC by GRNInfer, as shown in Figure 2.

Biological process enrichments (num.≥2) of high *E2F1* feedback-interactive *BRCA1* indirect pathway show feedback mitotic spindle assembly checkpoint of *TTK*, *CENPF*, regulation of cell cycle of *TTK*, *CENPF*; downstream G1/S transition of mitotic cell cycle of *MCM4*, *BCAT1*, small molecule metabolic process of *BCAT1*, *TK1*, metabolic pathways of *BCAT1*, *TK1* in HCC by DAVID and MAS 3.0. as shown in Table 1.

Knowledge enrichments (num.≥2) of the corresponding high *BRCA1* indirect molecular pathway demonstrate BM CD71 + early erythroid_3rd of upstream *CCNB2*, *LAPTM4B*, *NUSAP1*, feedback *CENPF*, *HIST1H2BJ*, *TTK*, *UNG*, downstream *BCAT1*, *HIST1H2AD*, *MCM4*, *TK1*; BM CD33 + Myeloid_3rd of upstream *CCNB2*, *NUSAP1*, feedback *CENPF*, *HIST1H2BJ*, *TTK*, *UNG*, downstream *BCAT1*, *HIST1H2AD*, *MCM4*, *TK1*; CD4+T cells_3rd of upstream *CCNB2*, *NUSAP1*, feedback *CENPF*, *TTK*, downstream *MCM4*, *TK1* in HCC based on GO, KEGG, BioCarta, GNF_U133A, UNIGENE_EST, Disease, GenMAPP databases by DAVID and MAS 3.0, as shown in Table 1.

Gene name of the corresponding high *BRCA1* indirect molecular pathway shows: (i) upstream lysosomal protein transmembrane 4 beta (LAPTM4B), or cyclin B2 (CCNB2)—nucleolar and spindle associated protein 1 (NUSAP1) activation; (ii) feedback uracil DNA glycosylase (UNG), or TTK protein kinase (TTK)—histone cluster 1, H2bj (HIST1H2BJ)—centromere protein F (CENPF); and (iii) downstream minichromosome maintenance complex component 4 (MCM4) or branched chain amino-acid transaminase 1, cytosolic (BCAT1)—histone

cluster 1, H2ad (HIST1H2AD) to thymidine kinase 1, soluble (TK1) in HCC by integration of GRNInfer with DAVID, as shown in Table 2.

## 4 | DISCUSSION

Based on regulation of cell cycle of *TTK*, *CENPF*, small molecule metabolic process of *BCAT1*, *TK1* in enrichments (num.≥2) of high *E2F1* feedback-interactive *BRCA1* indirect pathway by DAVID and MAS 3.0 (Table 1), biological process non-enrichments (num.=1) of the corresponding high *BRCA1* indirect pathway were identified as upstream regulation of cell cycle of *CCNB2*; feedback carbohydrate metabolism of *UNG*; downstream cellular nitrogen compound metabolic process of *BCAT1*, 2 Oxocarboxylic acid metabolism of *BCAT1*, nucleobase containing compound metabolic process of *TK1*, pyrimidine nucleobase metabolic process of *TK1*, thymidine metabolic process of *TK1*, nucleobase containing small molecule metabolic process of *TK1*, Pyrimidine metabolism of *TK1*, Drug metabolism other enzymes of *TK1* in HCC (Supplement S5). Therefore, we propose that *E2F1* interactive with *BRCA1* indirect pathway induces two different small molecule metabolism or cell cycle regulation in HCC.

Based on GNF_U133A and UNIGENE_EST QUARTILE enrichments analysis of high *E2F1* feedback-interactive *BRCA1* indirect pathway by DAVID (Table 1), the corresponding high *BRCA1* cell cycle regulation molecular pathway was identified as CD4+T cells_3rd of upstream *CCNB2*, *NUSAP1*; feedback *CENPF*, *TTK*; downstream *MCM4*, *TK1* in HCC by hypothesis driven. Therefore, we put forward that *E2F1* interactive with *BRCA1* indirect pathway induces CD4+T-related cell cycle regulation in HCC.

Based on cellular component, molecular function, gene name analysis of high *E2F1* feedback-interactive *BRCA1* indirect molecular pathway by DAVID (Table 2), the corresponding *BRCA1* small molecule metabolism or CD4+T-related cell cycle regulation pathway was identified as: (i) upstream endomembrane system lysosomal protein transmembrane 4 beta (LAPTM4B), or membrane cyclin B2 (CCNB2)—cytoplasm nucleolar and spindle associated protein 1 (NUSAP1) activation; (ii) feedback mitochondrion uracil DNA glycosylase (UNG), or membrane TTK protein kinase (TTK)—cytoplasm histone cluster 1, H2bj (HIST1H2BJ)—cytosol centromere protein F (CENPF); (iii) downstream membrane minichromosome maintenance complex component 4 (MCM4) or mitochondrion branched chain amino-acid transaminase 1, cytosolic (BCAT1)—nucleosome histone cluster 1, H2ad (HIST1H2AD) to cytosol thymidine kinase 1, soluble (TK1) in HCC by hypothesis driven. We put forwards *E2F1* interactive with *BRCA1* indirect pathway induces small molecule metabolism or CD4 + T-related cell cycle regulation through two different mitochondrion to cytosol protein and DNA binding or membrane to cytosol ATP and microtubule binding in HCC.

Based on high *E2F1* feedback-interactive *BRCA1* direct and indirect molecular pathway by GRNInfer (Figures 1 and 2), the corresponding small molecule metabolism pathway was identified as upstream *LAPTM4B* activation, feedback *UNG*, downstream *BCAT1-HIST1H2AD-TK1*; the corresponding CD4+T-related cell cycle regulation pathway as upstream *CCNB2-NUSAP1* activation, feedback *TTK-HIST1H2BJ-CENPF*, downstream *MCM4-TK1* in HCC by hypothesis driven. *E2F1* has positive relationship with the above molecules and family members in the references. Such as, *E2F1* and *CENPA* were upregulated in EBV-transformed lymphoblasts (Dai et al., 2012). The dysregulated cell cycle pathway involving *TTK* and *E2F1* may play significant roles in laryngeal squamous cell carcinoma progression (Hui, Yang, Yang, Guo, & Jang, 2015). *E2F1* and *H3K9ac* are responsible for the transcriptional regulation of *DNMT1* in *BRCA1*-mutated breast cancer (Li et al., 2014). *E2F1* mediates increased expression of *NuSAP* in recurrent prostate cancer (Gulzar, McKenney, & Brooks, 2013). *E2F1*-induced upregulation of cyclin E1 levels are enhanced by inhibition of miR-15 expression (Ofir, Hacohen, & Ginsberg, 2011). Reduced expression of *E2F1* and thymidine kinase was accompanied with arsenic trioxide-decreased cell viability in lung adenocarcinoma (Lam, Li, Zheng, Leung, & Ho, 2014).

Besides disease enrichments (num.≥2) among the corresponding high *BRCA1* indirect feedback/up/downstream pathway of 3rd to adrenal tumor, breast (mammary gland) cancer, cervical tumor, colorectal tumor, gastrointestinal tumor, germ cell tumor, leukemia, non glioma (Table 2), disease enrichments (num.≥2) among non-enrichments of the corresponding high *BRCA1* indirect feedback/up/downstream pathway widely distribute 3rd to esophageal tumor, kidney tumor, normal, respiratory tract tumor, retinoblastoma, urinary bladder tumor, uterine tumor in HCC by DAVID and MAS 3.0 (Supplement S5). Therefore, our established novel *E2F1* feedback-interactive *BRCA1* indirect pathway in HCC may become common one of tumor and cancer. We will test whether the *E2F1/BRCA1* direct/indirect molecular pathways are similar accross different analyses and whether the conclusions could be generalized in other type of databases.

In summary, we propose that *E2F1* interactive with *BRCA1* pathway induces HCC two different small molecule metabolism or cell cycle regulation via mitochondrion or CD4+T to cytosol. Knowledge analysis reflects novel *E2F1* feedback-interactive *BRCA1* direct and indirect pathway. It will be very useful to develop a new route of identifying novel markers and potential drugs for prognosis, and therapy of cancer.

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## CONFLICTS OF INTEREST

The authors report no conflicts of interest in this work.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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