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The roles of cell cycle and BRCA1 in the DNA damage response.†

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Abstract: In cancer stem cells (CSCs), epithelial-mesenchymal transition (EMT) networks play an important role to acquisition of the drug resistance and cancer malignant feature. To reveal the network pathways in EMT and CSCs, gene expression in diffuse- and intestinal-type gastric cancer (GC) have been analyzed. A canonical pathway on Cell Cycle: G_1/S Checkpoint Regulation was activated in diffuse-type GC, and Cyclins and Cell Cycle Regulation was activated in intestinal-type GC. Canonical pathway related to Role of BRCA1 in DNA Damage Response was activated in intestinal-type GC, where BRCA1 which is related to G_1/S phase transition was up-regulated. Cell cycle regulation may be altered in EMT condition.

Keywords: BRCA1; cancer stem cell; cell cycle; epithelial-mesenchymal transition; DNA damage response; molecular network

1. Introduction

In cancer stem cells (CSCs), epithelial-mesenchymal transition (EMT) networks play an important role to acquisition of the drug resistance and cancer malignant feature [1]. To reveal the network pathways in EMT and CSCs, gene expression in diffuse- and intestinal-type gastric cancer (GC) have been analyzed. Our previous findings identified several molecular networks and the related microRNAs (miRNAs) in intestinal- and diffuse-type GC [2-5]. Cell proliferation and regulation of the cell cycle are essential in cancer therapeutic targeting. In this article, we focus on the roles of cell cycle and BRCA1 in the DNA damage response in diffuse- and intestinal-type GC. Cell cycle regulation may play an important role in intestinal- and diffuse-type GC. The mechanism of cancer drug resistance would be highlighted by the involvement of cell cycle in EMT and CSCs.

2. Materials and Methods

2.1. RefSeq data analysis

The RefSeq data of diffuse-type and intestinal- GC are publicly available in The Cancer Genome Atlas (TCGA) of The cBioPortal for Cancer Genomics database [6-8] in NCI Genomic Data Commons (GDC) Data Portal [9]. From the data of stomach adenocarcinoma (TCGA, PanCancer Atlas), intestinal- and diffuse-type GC data, which are noted as chromosomal instability (CIN) and genomically stable (GS), respectively, in TCGA Research Network publication, were compared [8].

2.2. Molecular genome network analysis

Data of intestinal- and diffuse-type GC in TCGA cBioPortal Cancer Genomics were uploaded and analyzed through the use of Ingenuity Pathway Analysis (IPA) (QIAGEN Inc., Hilden, Germany) [10].

2.3. Data Visualization

The results of gene expression data of RefSeq and network analysis were visualized by Tableau software.

3. Results and Discussion

3.1. Canonical pathways altered in diffuse- and intestinal-type GC

Canonical pathways altered in diffuse- and intestinal-type GC are shown in Figure 1 and Table 1. The 2815 IDs which are significantly different in diffuse- and intestinal-type GC were analyzed in network analysis, which identified 69 canonical pathways related to the diffuse- and intestinal-type GC. Gene expression data of the diffuse- and intestinal-type GC revealed 36 canonical pathways with activation z-score as shown in Table 1. These canonical pathways include Cell Cycle: G_1/S Checkpoint Regulation, Cyclins and Cell Cycle Regulation, Role of BRCA1 in DNA Damage Response, and Cell Cycle: G_2/M DNA Damage Checkpoint.

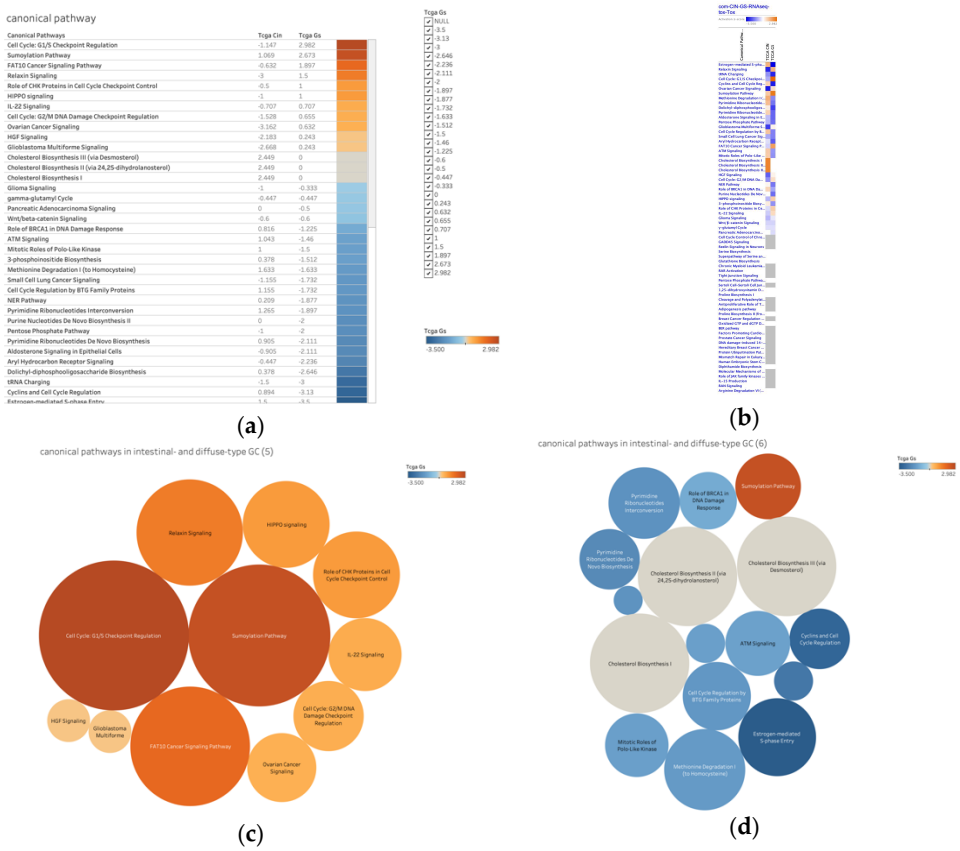


Figure 1. Canonical pathways altered in diffuse- and intestinal-type GC are shown. (**a**) Canonical pathways sorted by the order of the activation z-score in diffuse-type GC are shown; (**b**) Canonical pathways sorted by the order of the p-value of the pathways are shown; (**c**) Size and color indicate the activation z-score in diffuse-type GC; (**c**) Size indicates the activation z-score in intestinal-type GC and color indicates the activation z-score in intestinal-type GC.

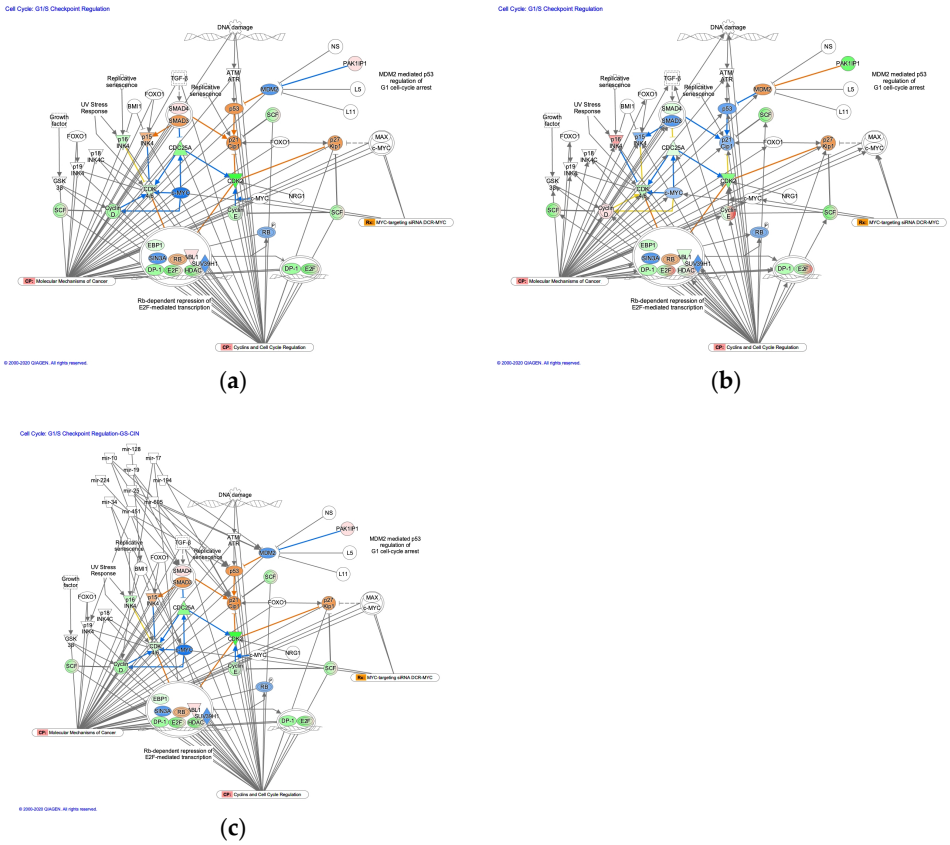
Table 1. Canonical pathways altered in diffuse- and intestinal-type GC. The pathways are sorted by the order of the activation z-score.

Canonical Pathways	**diffuse-type GC (activation z-score)**	**intestinal-type GC (activation z-score)**
Cell Cycle: G_1/S Checkpoint Regulation	2.982	-1.147
Sumoylation Pathway	2.673	1.069
FAT10 Cancer Signaling Pathway	1.897	-0.632
Relaxin Signaling	1.5	-3
HIPPO signaling	1	-1
Role of CHK Proteins in Cell Cycle Checkpoint Control	1	-0.5
IL-22 Signaling	0.707	-0.707
Cell Cycle: G_2/M DNA Damage Checkpoint Regulation	0.655	-1.528
Ovarian Cancer Signaling	0.632	-3.162
Glioblastoma Multiforme Signaling	0.243	-2.668
HGF Signaling	0.243	-2.183
Cholesterol Biosynthesis II (via 24,25-dihydrolanosterol)	0	2.449
Cholesterol Biosynthesis III (via Desmosterol)	0	2.449
Cholesterol Biosynthesis I	0	2.449
Glioma Signaling	-0.333	-1
gamma-glutamyl Cycle	-0.447	-0.447
Pancreatic Adenocarcinoma Signaling	-0.5	0
Wnt/beta-catenin Signaling	-0.6	-0.6
Role of BRCA1 in DNA Damage Response	-1.225	0.816
ATM Signaling	-1.46	1.043
Mitotic Roles of Polo-Like Kinase	-1.5	1
3-phosphoinositide Biosynthesis	-1.512	0.378
Methionine Degradation I (to Homocysteine)	-1.633	1.633
Small Cell Lung Cancer Signaling	-1.732	-1.155
Cell Cycle Regulation by BTG Family Proteins	-1.732	1.155
NER Pathway	-1.877	0.209
Pyrimidine Ribonucleotides Interconversion	-1.897	1.265
Pentose Phosphate Pathway	-2	-1

Purine Nucleotides De Novo Biosynthesis II	-2	0
Aldosterone Signaling in Epithelial Cells	-2.111	-0.905
Pyrimidine Ribonucleotides De Novo Biosynthesis	-2.111	0.905
Aryl Hydrocarbon Receptor Signaling	-2.236	-0.447
Dolichyl-diphosphooligosaccharide Biosynthesis	-2.646	0.378
tRNA Charging	-3	-1.5
Cyclins and Cell Cycle Regulation	-3.13	0.894
Estrogen-mediated S-phase Entry	-3.5	1.5

3.2. Cell Cycle: G1/S checkpoint Regulation pathway was activated in diffuse-type GC

Molecule activity predictor in IPA predicted the activation of Cell Cycle: G1/S Checkpoint Regulation pathway in diffuse-type GC (Figure 2). In Cell Cycle: G1/S Checkpoint Regulation pathway, DNA damage induces p53, which is expected to be activated in diffuse-type GC. Analysis of direct relationships of miRNAs and targeted molecules in Cell Cycle: G1/S Checkpoint Regulation pathway revealed the relationships between miRNAs and the targeted molecules (Figure 2c, Table 2).



(**a**) (**b**)

(**c**)

Figure 2. Cell Cycle: G1/S Checkpoint Regulation pathway was activated in diffuse-type GC. (**a**) Gene expression and pathway activity prediction in diffuse-type GC are shown; (**b**) Gene expression and pathway activity prediction in intestinal-type GC are shown; (**c**) Direct relationships of miRNAs and targeted molecules in the pathway are shown. The genes of which expression was altered in diffuse-

and intestinal-type GC are shown in pink (up-regulated) or green (down-regulated). Predicted activation or inhibition is shown in orange or blue, respectively.

Table 2. Direct relationships of miRNAs and targeted molecules in Cell Cycle: G1/S Checkpoint Regulation pathway.

From Molecule(s)	To Molecule(s)
mir-10	SMAD4
mir-10	SUV39H1
mir-10	p53
mir-128	BMI1
mir-17	CyclinD
mir-17	RB
mir-17	p21Cip1
mir-19	SMAD4
mir-19	p21Cip1
mir-194	MDM2
mir-224	SMAD4
mir-25	MDM2
mir-25	p21Cip1
mir-25	p53
mir-34	CDK4/6
mir-34	c-MYC
mir-34	p53
mir-451	p19INK4
mir-605	MDM2

3.3. Cyclins and Cell Cycle Regulation pathway was activated in intestinal-type GC

Molecule activity predictor in IPA predicted the activation of Cyclins and Cell Cycle Regulation pathway in intestinal-type GC (Figure 3). Analysis of direct relationships of miRNAs and targeted molecules in Cyclins and Cell Cycle Regulation pathway revealed the relationships between miRNAs and the targeted molecules (Figure 3c, Table 3).

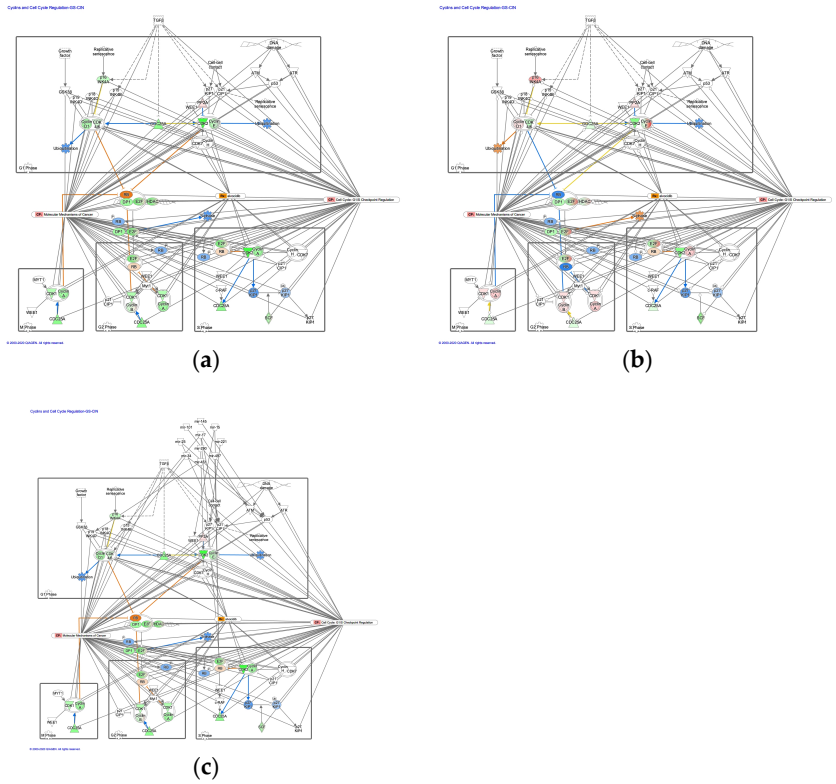


Figure 3. Cyclins and Cell Cycle Regulation pathway was activated in intestinal-type GC. (**a**) Gene expression and pathway activity prediction in diffuse-type GC are shown; (**b**) Gene expression and pathway activity prediction in intestinal-type GC are shown; (**c**) Direct relationships of miRNAs and targeted molecules in the pathway are shown. The genes of which expression was altered in diffuse- and intestinal-type GC are shown in pink (up-regulated) or green (down-regulated). Predicted activation or inhibition is shown in orange or blue, respectively.

Table 3. Direct relationships of miRNAs and targeted molecules in Cyclins and Cell Cycle Regulation pathway.

From Molecule(s)	To Molecule(s)
mir-101	ATM
mir-145	p53
mir-15	WEE1
mir-15	c-RAF
mir-17	ATM
mir-17	CyclinD1
mir-17	RB
mir-17	p21CIP1
mir-221	p27KIP1
mir-25	p21CIP1
mir-25	p53
mir-290	CDK2
mir-34	CDK4/6
mir-34	p53

mir-451	p19INK4D
mir-497	c-RAF

3.4. Role of BRCA1 in DNA Damage Response pathway was activated in intestinal-type GC

Molecule activity predictor in IPA predicted the activation of Role of BRCA1 in DNA Damage Response pathway in intestinal-type GC (Figure 4). Role of BRCA1 in DNA Damage Response pathway was identified as the most significant canonical pathway with p value of 6.6 x 10^{-12}. Gene expression of BRCA1 which is associated with G_1/S transition has increased in intestinal-type GC. BRCA1 codes a 190kD nuclear phosphorylation protein that maintains genomic stability and functions as a tumor suppressor. It is interesting that p53 and c21CIP1 are activate in intestinal-type GC in the Role of BRCA1 in DNA Damage Response pathway. BRCA1 may be involved in the activation of p53. Analysis of direct relationships of miRNAs and targeted molecules in Role of BRCA1 in DNA Damage Response pathway revealed the relationships between miRNAs and the targeted molecules (Figure 4c, Table 4). Ten miRNAs which have direct relationships between BRCA1 in Role of BRCA1 in DNA Damage Response pathway included miR-125a-3p (miRNAs w/seed CAGGUGA), miR-146a-5p (and other miRNAs w/seed GAGAACU), miR-224-5p (miRNAs w/seed AAGUCAC), miR-3615 (miRNAs w/seed CUCUCGG), miR-4639-3p (and other miRNAs w/seed CACUCUC), miR-5586-3p (miRNAs w/seed AGAGUGA), miR-6516-5p (miRNAs w/seed UUGCAGU), miR-6814-5p (miRNAs w/seed CCCAAGG), miR-6875-3p (miRNAs w/seed UUCUUCC), miR-99a-3p (and other miRNAs w/seed AAGCUCG) (Figure 4d, Table 5).

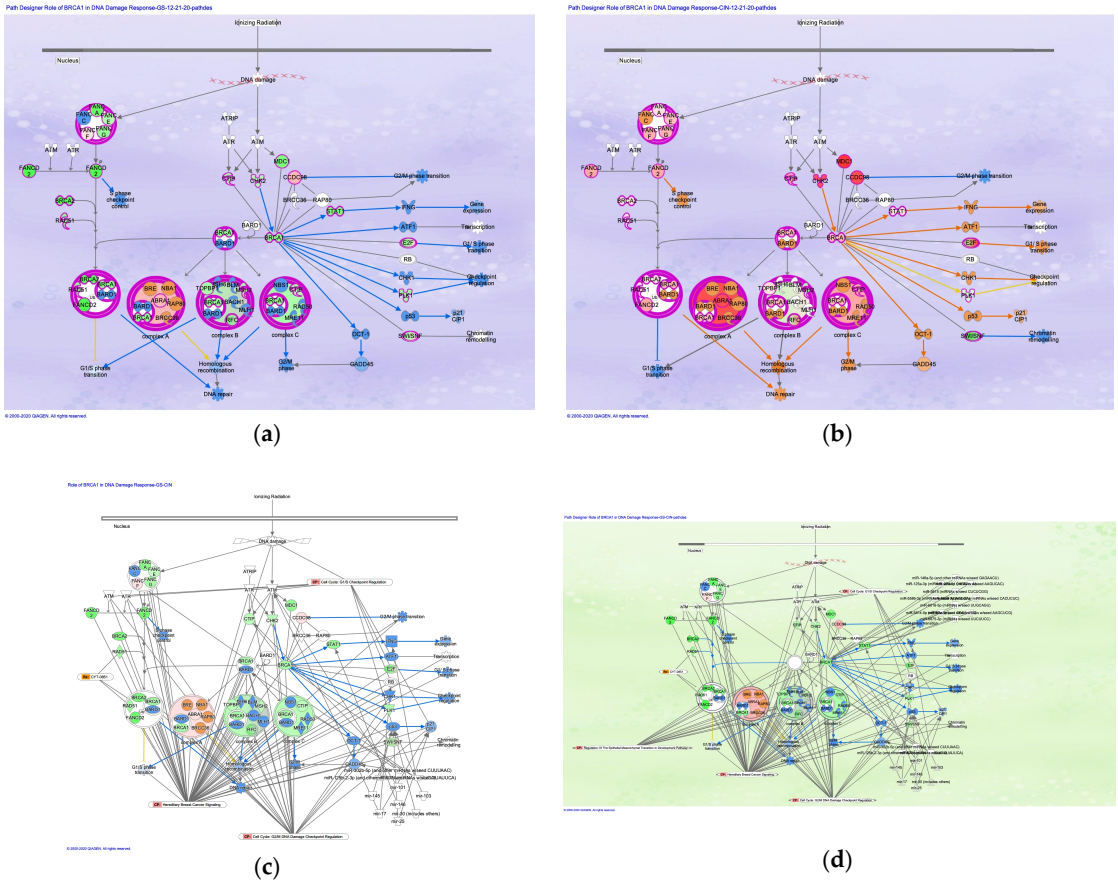


Figure 4. Role of BRCA1 in DNA Damage Response pathway was activated in intestinal-type GC. (**a**) Gene expression and pathway activity prediction in diffuse-type GC are shown; (**b**) Gene expression and pathway activity prediction in intestinal-type GC are shown; (**c**) Direct relationships of miRNAs and targeted molecules in the pathway are shown. (**d**) Direct relationships of miRNAs and BRCA1 in

the pathway in diffuse-type GC are shown. The genes of which expression was altered in diffuse- and intestinal-type GC are shown in pink (up-regulated) or green (down-regulated). Predicted activation or inhibition is shown in orange or blue, respectively.

Table 4. Direct relationships of miRNAs and targeted molecules in Role of BRCA1 in DNA Damage Response pathway.

From Molecule(s)	To Molecule(s)
miR-125b-2-3p (and other miRNAs w/seed CAAGUCA)	p53
miR-302b-5p (and other miRNAs w/seed CUUUAAC)	BARD1
miR-302b-5p (and other miRNAs w/seed CUUUAAC)	CTIP
miR-302b-5p (and other miRNAs w/seed CUUUAAC)	GADD45
miR-6074 (miRNAs w/seed AUAUUCA)	FANCF
miR-6074 (miRNAs w/seed AUAUUCA)	IFNG
miR-6074 (miRNAs w/seed AUAUUCA)	NBS1
mir-101	ATM
mir-103	p53
mir-145	p53
mir-146	STAT1
mir-17	ATM
mir-17	RB
mir-17	p21CIP1
mir-25	p21CIP1
mir-25	p53
mir-30 (includes others)	p53

Table 5. Ten miRNAs which have direct relationships between BRCA1 in Role of BRCA1 in DNA Damage Response pathway.

Ten miRNAs which have direct relationships between BRCA1
miR-125a-3p (miRNAs w/seed CAGGUGA)
miR-146a-5p (and other miRNAs w/seed GAGAACU)
miR-224-5p (miRNAs w/seed AAGUCAC)
miR-3615 (miRNAs w/seed CUCUCGG)
miR-4639-3p (and other miRNAs w/seed CACUCUC)
miR-5586-3p (miRNAs w/seed AGAGUGA)
miR-6516-5p (miRNAs w/seed UUGCAGU)
miR-6814-5p (miRNAs w/seed CCCAAGG)
miR-6875-3p (miRNAs w/seed UUCUUCC)
miR-99a-3p (and other miRNAs w/seed AAGCUCG)

3.5. Cell Cycle: G2/M DNA Damage Checkpoint Regulation pathway in diffuse- and intestinal- type GC

Cell Cycle: G2/M DNA Damage Checkpoint Regulation pathway was identified as related canonical pathway in diffuse- and intestinal- type GC (Figure 5). Analysis of direct relationships of miRNAs and targeted molecules in Cell Cycle: G2/M DNA Damage Checkpoint Regulation pathway revealed the relationships between miRNAs and the targeted molecules (Figure 5c, Table 6).

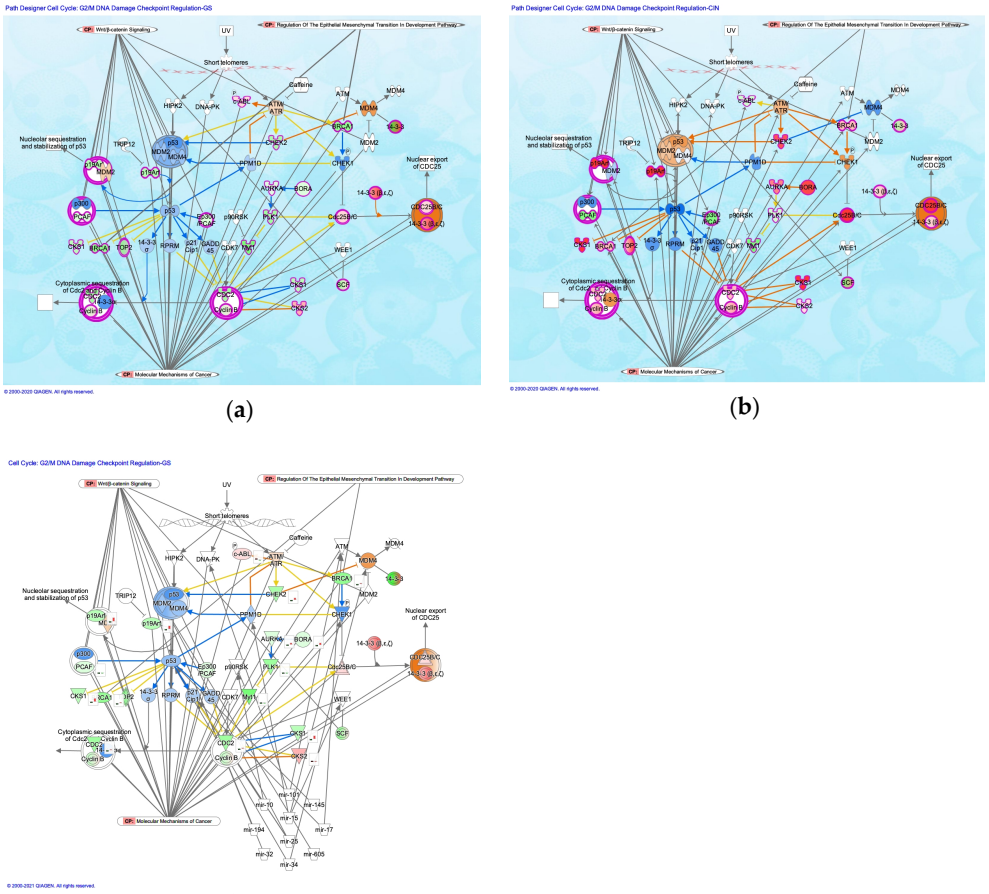


Figure 5. Cell Cycle: G2/M DNA Damage Checkpoint Regulation pathway in diffuse- and intestinal-type GC. (**a**) Gene expression and pathway activity prediction in diffuse-type GC are shown; (**b**) Gene expression and pathway activity prediction in intestinal-type GC are shown; (**c**) Direct relationships of miRNAs and targeted molecules in the pathway are shown. The genes of which expression was altered in diffuse- and intestinal-type GC are shown in pink (up-regulated) or green (down-regulated). Predicted activation or inhibition is shown in orange or blue, respectively.

Table 6. Direct relationships of miRNAs and targeted molecules in Cell Cycle: G2/M DNA Damage Checkpoint Regulation pathway.

From Molecule(s)	To Molecule(s)
mir-10	p53
mir-10	p90RSK
mir-101	ATM
mir-101	DNA-PK
mir-145	p53

mir-15	CHEK1
mir-15	PPM1D
mir-15	WEE1
mir-17	ATM
mir-17	p21Cip1
mir-194	MDM2
mir-25	MDM2
mir-25	p21Cip1
mir-25	p53
mir-32	MDM2
mir-34	MDM4
mir-34	p53
mir-605	MDM2

4. Conclusion

The several canonical pathways have been found to be altered in diffuse- and intestinal-type GC. Canonical pathway on Cell Cycle: G_1/S Checkpoint Regulation was activated in diffuse-type GC, and Cyclins and Cell Cycle Regulation was activated in intestinal-type GC. Canonical pathway related to Role of BRCA1 in DNA Damage Response was activated in intestinal-type GC, where BRCA1 which is related to G_1/S phase transition was up-regulated. Cell cycle regulation may be altered in EMT condition in diffuse-type GC.

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Conflicts of Interest: The authors declare no conflict of interest.

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