DNA Replication Inhibitor Geminin and Retinoic Acid Signaling Participate in Complex Interactions Associated With Pluripotency

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Abstract. Background/Aim: Several links between DNA replication, pluripotency and development have been recently identified. The involvement of miRNA in the regulation of cell cycle events and pluripotency factors has also gained attention. Materials and Methods: In the present study, we used the g:Profiler platform to analyze transcription factor binding sites, miRNA networks and protein-protein interactions to identify novel links among the aforementioned processes. Results and Conclusion: A complex circuitry between retinoic acid signaling, SWI/SNF components, pluripotency factors including Oct4, Sox2 and Nanog and cell cycle regulators was identified. It is suggested that the DNA replication inhibitor geminin plays a central role in this circuitry.

The maintenance of genome stability in living cells is associated with the tight regulation of DNA replication and integrity, so that the genome is fully and accurately replicated during each cell cycle. In eukaryotes, the initial steps of replication consist of the sequential assembly of pre-replicative complex (pre-RC) proteins onto the origins of replication. This process is named replication licensing and takes place during a restricted window of time from late mitosis to early G1 (1, 2). The pre-RCs consist of several proteins, including ORC, Cdt1, Cdc6 and MCM 2-7. Restriction of replication licensing from the end of mitosis to early G1 occurs by regulating Cdt1 levels, either by ubiquitin-mediated degradation of Cdt1 or inhibition by geminin (3). Geminin plays a central role in preventing DNA re-replication, a process that can lead to genomic instability and cancer development (3-5).

Geminin is a small nuclear protein (~25 kDa) that plays a critical role in cell cycle regulation by inhibiting DNA replication (6, 7). Geminin binds to and inhibits the DNA replication factor Cdt1. It is expressed in the S and G2 phases of the cell cycle and is degraded by the anaphase-promoting complex during the metaphase-anaphase transition (8).

Geminin has been found to up-regulate transcription of the geminin gene, suggesting that its expression may be regulated by a molecular feedback loop (9). Although GMNN is transcriptionally regulated by E2F family members, the mechanism by which geminin modulates E2F-mediated transcriptional regulation of the GMNN gene is not fully understood (10). Geminin ablation has been reported to enhance colon and lung carcinogenesis (4) while it has also been found to be overexpressed in several human cancers including colon, rectal, oral and breast cancer (11-13).
Similarly to other pre-RC components, geminin has been implicated in development and differentiation (14-16). In Xenopus embryos, it has been shown to induce cell differentiation contributing to the formation of the neural tube (17), while it has also been found to regulate the Hox homeobox proteins, controlling differentiation and proliferation (18). In another study with embryonic stem cells, geminin ablation was found to lead to loss of pluripotency and mesendodermal differentiation (19).

In the present article, we explored the interplay that seems to link the areas of DNA replication, pluripotency, development and cancer (14, 15, 20-22). Our main focus was to identify common regulatory nodes among networks of pluripotency and oncogenic factors, development and components of DNA replication. In this direction, we re-examined recent experimental data, in conjunction with in silico predictions placing retinoic acid and geminin on the forefront of this network.

Materials and Methods

The web-based g:GOSt tool from the g:Profiler platform was used to identify functional information and enriched pathways and processes from gene lists (23-25). Data for predictions of transcription factor binding sites were derived from the TRANSFAC database (26), protein-protein interactions from the BioGRID database (27) and miRNA target sites from the miRBase database (28). In all cases, multiple testing correction was performed using the g:SCS algorithm that is the default and most stringent algorithm (23, 24) in order to identify potential shared transcription factor (TF) binding sites from the TRANSFAC database (26). Interestingly, the Oct4 and geminin genomic loci were predicted to have binding sequences for the retinoic acid receptor (RAR) (p=0.016; g:SCS algorithm) (Figure 1), which is a TF as well as a nuclear receptor (32).

Retinoic acid (RA) has been reported to inhibit Oct4 expression during embryonic stem (ES) cell differentiation indirectly, by repressing a cis enhancer element (33), as well as silencing its promoter (34). However, in these experiments, the role of RAR in mediating the RA effects was not assessed.

RAR has been reported to modulate the expression of c-myc as well as several Hox genes (including HoxB4, HoxB7, HoxA9 and HoxA10) (35), while our recent microarray data have shown that geminin ablation in the murine haematopoietic system results in significant RAR up-regulation (36, 37). Interestingly, RA has also been shown to suppress Nanog, Oct4, geminin and Hox gene expression; however, the exact mechanism and whether it acts directly or indirectly, through RAR and/or other factors, is not known (Figure 1). More importantly, in a recent study, RA was reported to induce chromatin remodeling close to the Oct4 and Nanog genes and suppress their expression. This effect was dependent on a complex of RAR, receptor-interacting protein 140 (RIP140) and Brm. Using chromatin immunoprecipitation, the authors showed that Brm replaces another SWI/SNF subunit, Brg1, in this complex upon RA-induced repression, in the promoters of the aforementioned genes (38). In accordance with these data, Flajollet et al. (39) have also shown that RAR physically interacts with Brg1, as well as the SMARCD3/BAF606 complex, a core SWI/SNF subunit, which was eventually identified as a co-activator for RAR-induced transcription (Figure 1).

An interesting point is that during neural development, geminin has also been shown to directly interact with Brg1 and antagonize its activity, in order to maintain the cells in a multipotent state (29, 40, 41). Adding another layer of complexity, geminin is also known to interact with Hox genes, both directly and indirectly, through Polycomb (18, 36) (Figure 1) while BRG1 is known to control Nanog transcription through histone deacetylation (42) and occupy the promoters of Oct4, Sox2 and Nanog (43).
Moreover, RA has been shown to repress canonical and activate the non-canonical Wnt pathway in ES cells (44) and, in line with this, there is evidence that geminin expression is also regulated by Wnt. More specifically, geminin 5' regulatory sequences and endogenous geminin positively feedback to exogenous Wnt signals in Xenopus laevis embryos (45) while geminin down-regulation was shown to enhance Wnt signaling (46). This complex signaling cascade is summarized in Figure 1.

Evidence for Oct4 and geminin regulation by HNF4 and COUPTF. The results of the present study predict that, Oct4 and geminin, apart from RAR, have common binding sequences for HNF4 (p=0.016; g:SCS algorithm) and COUPTF (p=0.0266;
Common regulation of Oct4 and geminin by HNF4 seems to be in line with the recent finding that geminin together with the GATA6 TF can induce the generation of induced-pluripotent stem cells (iPSCs), without the need for Oct4 and Sox2 expression (47). Interestingly, our previous RNA-seq has shown that upon geminin ablation, HNF4a is highly up-regulated in the fetal liver (36).

Additionally, there is experimental evidence that COUP-TF is a ligand-activated nuclear receptor, with RA as a ligand (48), while other studies had shown that this receptor serves as a RAR accessory protein (49) and is involved in RA signaling (50-52). Interestingly, a regulatory network has also been identified, involving the miRNA miR-302 and the TFs OCT4 and COUPTFII (53) (Figure 1).

Geminin, miRNAs, GABA signaling and retinoic acid

Recent data have revealed an important role for miRNAs in pluripotency as well as regulation of the cell cycle. miRNAs can maintain the pluripotency state (54) or facilitate an exit, by repressing core pluripotency factors (55, 56). There is also increasing evidence about their role in the cell cycle and replicative stress (57, 58). It has been shown, for example, that the miR-34 family targets the MCM proteins of the pre-RC complex (59-61).

Geminin and mir-452. Geminin has only recently been reported to be targeted by miR-571, the only miRNA known to date to prevent aberrant DNA replication (62). Besides MiR-571, no other miRNA has been reported to target geminin or any pre-RC component associated with the previously described circuitry. Nevertheless, geminin appears to share a spatiotemporal expression pattern with mir-452.

Firstly, this miRNA is enriched during mouse neural crest development where it plays a role in the epithelial-mesenchymal signaling; mir-452 down-regulation affects the Sonic hedgehog and Fgf8 signaling in the first branchial arch, through Wnt5a down-regulation, resulting in craniofacial defects (63). Similarly, a study by Emmett and O’Shea has shown that geminin knockdown resulted in E9.5 embryos with smaller and abnormally oriented first branchial arch with reduced Fgf8 expression (64). In line with this, our previous results have shown that mice lacking geminin expression have a reduced number of neural crest cells at E9.5 and 10.5 (65). Another study has reported similar results by E10.5 (66), whereas, in a reciprocal approach, FGF8 has been reported to induce geminin expression (30). Geminin down-regulation has also been reported to up-regulate Wnt5a in the primitive streak (46) and has been associated to the epithelial-mesenchymal transition (EMT), even though there is conflicting evidence as to whether its down-regulation (46) or overexpression (64, 67) promotes EMT.

Secondly, mir-452 overexpression has been reported to down-regulate the pluripotency regulators Klf4, Sox2, Oct4, Nanog and c-Myc as well as Bmi1, LEF1 and TCF4 in...
As well as geminin and Mcm2. According to similar and Rex1, as well as Brg1, HoxC13 and Klf4. More to be coregulated with pluripotency factors Nanog, Oct4, Sox2 factors specifically, mmu-miR-883b-5p is predicted to bind to Nanog.

hsa-miR-367 binds to Nanog as well as Cdc6 and Orc1. hsa-predictions, mmu-miR-706 binds to Oct4, Orc1 and c-myc. hsa-miR-423-5p binds to HoxC13, Brg1 and Mcm2. hsa-miR-452 binds to Klf4, Brg1 and Rex1. All the above including some further predictions are graphed as a network in Figure 2.

Several of these miRNAs have been experimentally reported to be modulated by retinoic acid. let-7b, predicted to bind to Sox2, geminin and CDC6 UTRs, has been found to be up-regulated in response to all-trans retinoic acid treatment of the NB4 cells, a human acute promyelocytic leukemia cell line (84). Similarly, miR-883b-5p, predicted to bind to Nanog, MCM2 and geminin UTRs, has been found to be highly up-regulated in J1 mouse ES cells upon RA-induced differentiation (85), while miR-423, predicted to bind to HoxC13, Brg1 and MCM2 was up-regulated in the neuroblast-like SH-SY5Y cells, again, upon RA induction (86). In the latter cell line, RA has also been reported to up-regulate miR-628-3p (predicted to bind to the UTRs of geminin, ORC1 and CDC6) and down-regulate miR-490-3p (predicted to bind to CDC6, ORC1 and Rex1) (87).

**Conclusion**

Based on the results of the present study, along with extensive evidence from the literature, it is evident that there is a circuitry between RA signaling, SWI/SNF, pluripotency factors and cell-cycle regulators. The role of geminin in this circuitry is shown to be of great significance.

While being essential for the maintenance of genome stability, we have previously shown that geminin acts as a tumor suppressor in the murine colon and lung cancer model (4). In addition, it is frequently overexpressed in several human cancers and a recent study has shown that geminin overexpression promotes breast cancer metastasis through FoxO3 deacetylation (88). Geminin is, therefore, involved in cancer, development and pluripotency. It has also recently been reported to be targeted by miR-571, the first miRNA to prevent aberrant DNA replication (62).

Further transcriptional and miRNA interactions could be examined by molecular dynamic simulations (89-92) and verified _in vitro_ by chromatin immunoprecipitation, miRNA/ mRNA co-expression and the study of miRNA effects on target proteins (93), along with analysis of possible epigenetic changes. A better understanding of this crosstalk will be invaluable for delineating the cell-cycle links to the loss of pluripotency, subsequent cell differentiation and oncogenesis.

**Conflicts of Interest**

The Authors declare no conflicts of interest regarding this study.
Authors’ Contributions

SCT and ST designed the study and SCT wrote the paper. GJD and MP wrote portions of the paper. SCT and DV performed the bioinformatic analysis. SCT, GJD, VB, GTS and ST analyzed the data relating to transcription factor binding sites. SCT, AP, AKA, MV and GTS analyzed the data relating to miRNA interactions. All authors critically reviewed the final version of the paper.

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References

1 Symeonidou IE, Kotsantis P, Roukos V, Rapsomakini MA, Grecco HE, Bastaens P, Taraviras S and Lygerou Z: Multi-step loading of human minichromosome maintenance proteins in live human cells. J Biol Chem 288: 35852-35867, 2013. PMID: 24158436. DOI: 10.1074/jbc.M113.474825

2 Fragkos M, Gavier O, Coulombe P and Mecalli M: DNA replication origin activation in space and time. Nat Rev Mol Cell Biol 16: 360-374, 2015. PMID: 25999062. DOI: 10.1038/nrm4002

3 Petropoulos M, Champeris Tsaniras S, Taraviras S and Lygerou Z: Replication licensing aberrations, replication stress, and genomic instability. Trends Biochem Sci 44(9): 752-764, 2019. PMID: 31054805. DOI: 10.1016/j.tibs.2019.03.011

4 Champeris Tsaniras S, Villiou M, Giannou AD, Nikou S, Petropoulos M, Pateras IS, Tserou P, Karousi F, Lalioti ME, Gorgoulis VG, Patmanidi AL, Stathopoulos GT, Bravou V, Lygerou Z and Taraviras S: Geminin ablation in vivo enhances tumorigenesis through increased genomic instability. J Pathol 246: 134-140, 2018. PMID: 29952003. DOI: 10.1002/path.5128

5 Gorgoulis VG, Vassilou LVF, Karakaidos P, Zacharakos P, Kotsinas A, Liloglou T, Venere M, DiTullio RA, Kastrinakis NG, Levy B, Kletsas D, Yoneta A, Herlyn M, Kittas C and Halazonetis TD: Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 434: 907-913, 2005. PMID: 15874965. DOI: 10.1038/nature03845

6 Montanari M, Maclaluso A, Cittadini A and Giordano A: Role of repressor: from normal control of DNA replication to cancer. Cell Death Differ 13: 1052-1056, 2006. PMID: 16628231. DOI: 10.1038/sj.cdd.4401932

7 Petropoulos C, Kotantaki P, Karamitros D and Taraviras S: Cdt1 and Geminin in cancer: markers or triggers of malignant transformation? Front Biosci 13: 4485-4494, 2008. PMID: 18508524. DOI: 10.2741/3018

8 McGarry T and Kirschner M: Geminin, an inhibitor of DNA replication, is degraded during mitosis. Cell 93: 1043-1053, 1998. PMID: 9635433. DOI: 10.1016/s0092-8674(00)81209-x

9 Ohno Y, Saeki K, Yasunaga S, Kurogi T, Suzuki-Takedachi K, Shirai M, Mihara K, Yoshida K, Voncken J, Ohtsubo M and Takiyara Y: Transcription of the Geminin gene is regulated by a negative-feedback loop. Mol Biol Cell 25: 1374-1383, 2014. PMID: 24554762. DOI: 10.1091/mbc.E13-09-0534

10 Yoshida K and Inoue I: Regulation of Geminin and Cdt1 expression by E2F transcription factors. Oncogene 23: 3802-3812, 2004. PMID: 14990995. DOI: 10.1038/sj.onc.1207488

11 Bravou V, Nishitani H, Song SY, Taraviras S and Varaklis J: Expression of the licensing factors, Cdt1 and Geminin, in human colon cancer. Int J Oncol 27: 1511-1518, 2005. PMID: 16273206.

12 Blanchard Z, Malik R, Mullins N, Maric C, Luk H, Horio D, Hernandez B, Killeen J and ElShamy WM: Geminin overexpression induces mammary tumors via suppressing cytokinesis. Oncotarget 2: 1011-1027, 2011. PMID: 22184288. DOI: 10.18632/oncotarget.363

13 Siril Y, Kouketsu A, Oikawa M, Takahashi T and Kumatomo H: Immunohistochemical assessment of chromatin licensing and DNA replication factor 1, geminin, and γ-H2AX in oral epithelial precursor lesions and squamous cell carcinoma. J Oral Pathol Med, 2019. PMID: 31318980. DOI: 10.1111/jop.12925

14 Champeris Tsaniras S, Kanelakis N, Symeonidou IE, Nikolopoulos P, Lygerou Z and Taraviras S: Licensing of DNA replication, cancer, pluripotency and differentiation: An interlinked world? Semin Cell Dev Biol 30: 174-180, 2014. PMID: 24641889. DOI: 10.1016/j.semcdb.2014.03.013

15 Champeris Tsaniras S, Vlachakis D and Taraviras S: The Nucleophosmin-Pin1 interaction links the cell cycle, cancer and pluripotency. J Mol Biochem 4: 50-51, 2015.

16 Patmanidi AL, Champeris Tsaniras S, Karamitros D, Kyrouci C, Lygerou Z and Taraviras S: Concise review: Geminin-A tale of two tails: DNA replication and transcriptional/epigenetic regulation in stem cells. Stem Cells 35: 299-310, 2017. PMID: 27859962. DOI: 10.1002/stem.2529

17 Kroll K, Salic A, Evans L and Kirschner M: Geminin, a neuralizing molecule that demarcates the future neural plate at the onset of gastrulation. Development 125: 3247-3258, 1998. PMID: 9671596.

18 Luo L, Yang X, Takiyara Y, Knoetgen H and Kessel M: The cell-cycle regulator geminin inhibits Hox function through direct and polycistrn-mediated interactions. Nature 427: 749-753, 2004. PMID: 14973489. DOI: 10.1038/nature02305

19 Tabrizi GA, Böse K, Reimann Y and Kessel M: Geminin is required for the maintenance of pluripotency. PLoS One 8: e73826, 2013.

20 Zhao X, Ji J, Yu L-R, Veenstra T and Wang XW: Cell cycle-dependent phosphorylation of nucleophosmin and its potential regulation by peptide-prolyl cis/trans isomerase. J Mol Biochem 4: 95-103, 2015. PMID: 27099843.

21 Kareta MS, Sage J and Wernig M: Crosstalk between stem cell and cell cycle machineries. Curr Opin Cell Biol 37: 68-74, 2015. PMID: 26520682. DOI: 10.1016/j.cceb.2015.10.001

22 Gonzales KAU, Liang H, Lim YS, Chan YS, Yeo JC, Tan CP, Gao B, Le B, Tan ZY, Low KY, Liou YC, Bard F and Ng HH: Deterministic restriction on pluripotent state dissolution by cell-cycle pathways. Cell 162: 564-579, 2015. PMID: 26232226. DOI: 10.1016/j.cell.2015.07.001

23 Reimand J, Kull M, Peterson H, Hansen J and Vilo J: g:Profiler—a web-based toolset for functional profiling of gene lists from large-scale experiments. Nucleic Acids Res 35: W193-200, 2007. PMID: 17478515. DOI: 10.1093/nar/gkm226

24 Reimand J, Arak T and Vilo J: g:Profiler – a web server for functional interpretation of gene lists (2011 update). Nucleic
Acids Res 39: W307-315, 2011. PMID: 21646343. DOI: 10.1093/nar/gkr378

Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H and Vilo J: g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res 47: W191-W198, 2019. PMID: 31066453. DOI: 10.1093/nar/gkz369

Matys V, Kel-Margoulis O V, Fricke E, Liebich I, Land S, Barre-Dirrie A, Reuter I, Chekmenev D, Krull M, Hornischer K, Voss N, Stegmaier P, Lewicki-Potapov B, Saxel H, Kel AE and Wingender E: TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. Nucleic Acids Res 34: D108-110, 2006. PMID: 16381825. DOI: 10.1093/nkj/dji43

Oughtred R, Stark C, Breitkreutz B-J, Rust J, Boucher L, Chang C, Kolas N, O’Donnell L, Leung G, McAdam R, Zhang F, Dolma S, Willems A, Couble-Huntington J, Chatr-Aryamontri A, Dolinski K and Tyers M: The BioGRID interaction database: 2019 update. Nucleic Acids Res 47: D529-D541, 2019. PMID: 30476227. DOI: 10.1093/nkj/dkj079

Griffiths-Jones S, Saini HK, Van Dongen S and Enright AJ: miRBase: tools for microRNA genomics. Nucleic Acids Res 36, 2008. PMID: 17991681. DOI: 10.1093/nkj/mkm952

Yang V, Carter S, Hyland S, Tachibana-Konwalski K, Laskey R and Gonzalez M: Geminin escapes degradation in G1 of mouse pluripotent cells and mediates the expression of Oct4, Sox2, and Nanog. Curr Biol 21: 692-699, 2011. PMID: 21497086. DOI: 10.1016/j.cub.2011.03.026

Papanayotou C, Mey A, Birot AM, Saka Y, Boast S, Smith JC, Blumberg B: ERF and ETV3L are retinoic acid-inducible repressors required for primary neurogenesis. Development 140: 3095-106, 2013. PMID: 23824578. DOI: 10.1242/dev.093716

Gutierrez-Mazariegos J, Schubert M and Laude T: Evolution of retinoic acid receptors and retinoic acid signaling. Subcell Biochem 70: 55-73, 2014. PMID: 24962881. DOI: 10.1007/978-94-017-9505-5_4

Okazawa H, Okamoto K, Ishino F, Ishino-Kaneko T, Toyoda Y, Muramatsu M and Hamada H: The oct3 gene, a gene for an embryonic transcription factor, is controlled by a retinoic acid repressible enhancer. EMBO J 10: 2997-3005, 1991. PMID: 1915274.

Schoorlemmer J, van Puinenbroek A, van Den Eijnden M, Jonk L, Dirrie A, Reuter I, Chekmenev D, Krull M, Hornischer K, Voss N, McAdam R, Zhang F, Dolma S, Willems A, Couble-Huntington J, Chatr-Aryamontri A, Dolinski K and Tyers M: The BioGRID interaction database: 2019 update. Nucleic Acids Res 47: D529-D541, 2019. PMID: 30476227. DOI: 10.1093/nkj/dkj079

Champeris Tsaniras S: Generating Mature β-Cells From Embryonic Stem Cells. Strategies for Late-Stage Differentiation. In: Vitamins and Hormones. Academic Press Inc., pp 79-92, 2011. PMID: 22127238. DOI: 10.1016/B978-0-12-386015-6.00025-1

Taylor JJ, Wang T and Kroll KL: Tcf- and Vent-binding sites regulate neural-specific geminin expression in the gastrula embryo. Dev Biol 289: 494-506, 2006. PMID: 16337935. DOI: 10.1016/j.ydbio.2005.10.047

Caronna EA, Patterson ES, Hummert PM and Kroll KL: Geminin restrains mesendodermal fate acquisition of embryonic stem cells and is associated with antagonism of Wnt signaling and enhanced polycomb-mediated repression. Stem Cells 31: 1477-1487, 2013. PMID: 23630199. DOI: 10.1002/stem.1410

Shu J, Wu C, Wu Y, Li Z, Shao S, Zhao W, Tang X, Yang H, Shen L, Zuo X, Yang W, Shi Y, Chi X, Zhang H, Gao G, Shu Y, Yuan K, He W, Tang C, Zhao Y and Deng H: Induction of pluripotency in mouse somatic cells with lineage specifiers. Cell 153: 23630199. DOI: 10.1002/stem.1410

Kroll KL: Geminin regulates neuronal differentiation by antagonizing Brg1 activity. Genes Dev 19: 1723-1734, 2005. PMID: 16024661. DOI: 10.1101/gad.1319105

Carey T, Cao Z, Choi I, Ganguly A, Wilson C, Paul S and Knott J: BRG1 Governs Nanog Transcription in Early Mouse Embryos and Embryonic Stem Cells via Antagonism of Histone H3 Lysine 9/14 Acetylation. Mol Cell Biol 35: 4158-4169, 2015. PMID: 26418882. DOI: 10.1128/MCB.00546-15

Kidder B, Palmer S and Knott J: SWI/SNF-Brg1 regulates self-renewal and occupies core pluripotency-related genes in embryonic stem cells. Stem Cells 27: 317-328, 2009. PMID: 19056910. DOI: 10.1634/stemcells.2008-0710

Champeris Tsaniras S: Geminin and Retinoic Acid Signaling Participate in Pluripotency-associated Interactions. Biochem Soc Trans 47: 4306-4317, 2014. PMID: 24489122. DOI: 10.1093/bst/oux092

Flajollet S, Lefebvre B, Cudejko C, Staels B and Lefebvre P: The core component of the mammalian SWI/SNF complex SMARCD3/BAF60c is a coactivator for the nuclear retinoic acid receptor. Mol Cell Endocrinol 270: 23-32, 2007. PMID: 17363140. DOI: 10.1016/j.mce.2007.02.004

Roukos V, Iliou MS, Nishitani H, Gentzel M, Wilm M, Taraviras S and Lengyel Z: Geminin cleavage during apoptosis by caspase-3 alters its binding ability to the SWI/SNF subunit Braham. J Biol Chem 282: 9346-9357, 2007. PMID: 17261582. DOI: 10.1074/jbc.M611643200

Seo S, Herr A, Lim J-W, Richardson GA, Richardson H and Kroll KL: Geminin regulates neuronal differentiation by antagonizing Brg1 activity. Genes Dev 19: 1723-1734, 2005. PMID: 16024661. DOI: 10.1101/gad.1319105

Patmanidi AL, Kanellakis NI, Karamitros D, Papadimitriou C, Lygerou Z and Taraviras S: Whole transcriptome data analysis of mouse embryonic hematopoietic stem and progenitor cells that lack Geminin expression. Data Br 7: 889-893, 2016. PMID: 27077091. DOI: 10.1016/j/db.2016.03.028

Kruse SW, Suino-Powell K, Zhou XE, Kretschman JE, Reynolds R, Vonrhein C, Xu Y, Wang L, Tsai SY, Tsai MJ and Xu HE: Identification of COP9-TFII orphan nuclear receptor as a retinoic acid-activated receptor. PLoS Bio 6: e227, 2008. PMID: 18798693. DOI: 10.1371/journal.pbio.0060227

Lin B, Chen GQ, Xiao D, Kolluri SK, Cao X, Su H and Zhang XK: Orphan receptor COUP-TF is required for induction of retinoic acid receptor beta, growth inhibition, and apoptosis by
retinoic acid in cancer cells. Mol Cell Biol 20: 957-970, 2000. PMID: 10629053. DOI: 10.1128/mcb.20.3.957-970.2000

50 Kliewer SA, Umesono K, Heyman RA, Mangelsdorf DJ, Dyck JA and Evans RM: Retinoid X receptor-COUP-TF interactions modulate retinoic acid signaling. Proc Natl Acad Sci USA 89: 1448-1452, 1992. PMID: 1311101. DOI: 10.1073/pnas.89.4.1448

51 Pickens BS, Teets BW, Sropano JR and Sropano DR: Role of COUP-TFII during retinoic acid-induced differentiation of P19 cells to endodermal cells. J Cell Physiol 228: 791-800, 2013. PMID: 23081528. DOI: 10.1002/jcp.24228

52 Love CE and Prince VE: Expression and retinoic acid regulation of the zebrafish miR-290 cluster modulates pluripotency by repressing canonical NF-xb signaling. Stem Cells 30: 655-664, 2012. PMID: 22232084. DOI: 10.1002/stem.1033

53 Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, Tian Y, Lüningschrör P, Stöcker B, Kaltschmidt B and Kaltschmidt C: Expression and retinoic acid regulation of the zebrafish nr2f orphan nuclear receptor genes. Dev Dyn 241: 1603-1615, 2012. PMID: 22836912. DOI: 10.1002/dvdy.23388

54 Xu N, Papagiannakopoulos T, Pan G, Thomson JA and Kosik KS: MicroRNA-145 Regulates OCT4, SOX2, and KLF4 and Represses Pluripotency in Human Embryonic Stem Cells. Cell Stem Cell 13: 67-65, 2009. PMID: 19496067. DOI: 10.1016/j.stem.2009.02.038

55 Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, Tian Y, Lüningschrör P, Stöcker B, Kaltschmidt B and Kaltschmidt C: Expression and retinoic acid regulation of the zebrafish nr2f orphan nuclear receptor genes. Dev Dyn 241: 1603-1615, 2012. PMID: 22836912. DOI: 10.1002/dvdy.23388

56 Xu N, Papagiannakopoulos T, Pan G, Thomson JA and Kosik KS: MicroRNA-145 Regulates OCT4, SOX2, and KLF4 and Represses Pluripotency in Human Embryonic Stem Cells. Cell Stem Cell 13: 67-65, 2009. PMID: 19496067. DOI: 10.1016/j.stem.2009.02.038

57 Bueno MJ and Malumbres M: MicroRNAs and the cell cycle. EMBO J 21: 791-800, 2002. PMID: 1177-1189, 2013. PMID: 23249188. DOI: 10.1038/scc.2012.050

58 Liu L, Chen K, Wu J, Shi L, Hu B, Cheng S, Li M and Song L: Downregulation of miR-452 promotes stem-like traits and tumorigenicity of gliomas. Clin Cancer Res 19: 3429-3438, 2013. PMID: 23695168. DOI: 10.1158/1078-0432.CCR-12-3794

59 Zheng Z, Liu J, Yang Z, Wu L, Xie H, Jiang C, Lin B, Chen T, Xing C, Liu Z, Song Y, Yin S, Zheng S and Zhou L: MicroRNA-452 promotes stem-like cells of hepatocellular carcinoma by inhibiting sox7 involving wnt/b-catenin signaling pathway. Oncotarget 7: 28000-28012, 2016. PMID: 27058905. DOI: 10.18632/oncotarget.8584

60 Knoll S, Fürst K, Kowtharapu B, Schmitz U, Marquardt S, Wolfenbauer O, Martin H and Pützer BM: E2F1 induces miR-224/452 expression to drive EMT through TNXIP downregulation. EMBO Rep 15: 1315-1329, 2014. PMID: 25341426. DOI: 10.15252/embr.201439392

61 Nie W, Huang W, Zhang W, Xu J, Song W, Wang Y, Zhu A, Luo J, Huang G, Wang Y and Guan X: TNXIP interaction with the Her-1/2 pathway contributes to overall survival in breast cancer. Oncotarget 6: 3003-3012, 2015. PMID: 25605021. DOI: 10.18632/oncotarget.3096

62 Zhao D, Besser AH, Wander SA, Sun J, Zhou W, Wang B, Ince T, Durante MA, Guo W, Mills G, Theodosiou D and Slingerland J: Cytoplasmic p27 promotes epithelial-mesenchymal transition and tumor metastasis via STAT3-mediated Twist1 upregulation. Oncogene 34: 5447-5459, 2015. PMID: 25684140. DOI: 10.1038/onc.2014.473

63 Andäng M, Hjerling-Leffler J, Moliner A, Lundgren TK, Castelo-Branco G, Nanou E, Pozas E, Bryja V, Halliez S, Nishimaru H, Ibañez CF and Ernfors P: Histone H2AX-dependent GABAA receptor regulation of stem cell proliferation. Nature 451: 460-464, 2008. PMID: 18185516. DOI: 10.1038/nature06488

64 Emmett LSD and O’Shea KS: Geminin is required for epithelial to mesenchymal transition at gastrulation. Stem Cells Dev 21: 2395-2409, 2012. PMID: 22335560. DOI: 10.1089/scd.2011.0483

65 Stathopoulou A, Natarajan D, Nikolopoulou P, Patmanidi AL, Lygerou Z, Pachnis V and Taraviras S: Inactivation of Geminin in neural crest cells affects the generation and maintenance of enteric progenitor cells, leading to enteric aganglionosis. Dev Biol 409: 392-405, 2016. PMID: 26658318. DOI: 10.1016/j.ydbio.2015.11.023

66 Patterson ES, Waller LE and Kroll KL: Geminin loss causes neural tube defects through disrupted progenitor specification and neuronal differentiation. Dev Biol 413: 44-56, 2014. PMID: 24995796. DOI: 10.1016/j.ydbio.2014.06.021

67 Slawny N and O’Shea KS: Geminin promotes an epithelial-to-mesenchymal transition in an embryonic stem cell model of gastrulation. Stem Cells Dev 22: 1177-1189, 2013. PMID: 21299481. DOI: 10.1089/scd.2012.0505

68 Liu L, Chen K, Wu J, Shi L, Hu B, Cheng S, Li M and Song L: Downregulation of miR-452 promotes stem-like traits and tumorigenicity of gliomas. Clin Cancer Res 19: 3429-3438, 2013. PMID: 23695168. DOI: 10.1158/1078-0432.CCR-12-3794

69 Zheng Z, Liu J, Yang Z, Wu L, Xie H, Jiang C, Lin B, Chen T, Xing C, Liu Z, Song Y, Yin S, Zheng S and Zhou L: MicroRNA-452 promotes stem-like cells of hepatocellular carcinoma by inhibiting sox7 involving wnt/b-catenin signaling pathway. Oncotarget 7: 28000-28012, 2016. PMID: 27058905. DOI: 10.18632/oncotarget.8584

60 Knoll S, Fürst K, Kowtharapu B, Schmitz U, Marquardt S, Wolfenbauer O, Martin H and Pützer BM: E2F1 induces miR-224/452 expression to drive EMT through TNXIP downregulation. EMBO Rep 15: 1315-1329, 2014. PMID: 25341426. DOI: 10.15252/embr.201439392

61 Nie W, Huang W, Zhang W, Xu J, Song W, Wang Y, Zhu A, Luo J, Huang G, Wang Y and Guan X: TNXIP interaction with the Her-1/2 pathway contributes to overall survival in breast cancer. Oncotarget 6: 3003-3012, 2015. PMID: 25605021. DOI: 10.18632/oncotarget.3096

62 Zhao D, Besser AH, Wander SA, Sun J, Zhou W, Wang B, Ince T, Durante MA, Guo W, Mills G, Theodosiou D and Slingerland J: Cytoplasmic p27 promotes epithelial-mesenchymal transition and tumor metastasis via STAT3-mediated Twist1 upregulation. Oncogene 34: 5447-5459, 2015. PMID: 25684140. DOI: 10.1038/onc.2014.473

63 Andäng M, Hjerling-Leffler J, Moliner A, Lundgren TK, Castelo-Branco G, Nanou E, Pozas E, Bryja V, Halliez S, Nishimaru H, Wilbertz J, Arenas E, Koltzenburg M, Charnay P, Márquez E, Pozas E, Bryja V, Halliez S, Nishimaru H, Wilbertz J, Are...
rereplication in the presence of functional p53. J Cell Biol 165: 473-482, 2004. PMID: 15159417. DOI: 10.1083/jcb.200403106

76 Zhu W, Chen Y and Dutta A: Rereplication by depletion of geminin is seen regardless of p53 status and activates a G2/M checkpoint. Mol Cell Biol 24: 7140-7150, 2004. PMID: 15282313. DOI: 10.1128/MCB.24.16.7140-7150.2004

77 Barry KA, Schultz KM, Payne CJ and McGarry TJ: Geminin is required for mitotic proliferation of spermatogonia. Dev Biol 371: 35-46, 2012. PMID: 22898305. DOI: 10.1016/j.ydbio.2012.07.031

78 de Renty C, Kaneko KJ and DePamphilis ML: The dual roles of geminin during trophoblast proliferation and differentiation. Dev Biol 387: 49-63, 2014. PMID: 24412371. DOI: 10.1016/j.ydbio.2013.12.034

79 Karamitros D, Kotantaki P, Lygerou Z, Veiga-Fernandes H, Pachnis V, Kioussis D and Taraviras S: Differential geminin requirement for proliferation of thymocytes and mature T cells. J Immunol 184: 2432-2441, 2010. PMID: 20107189. DOI: 10.4049/jimmunol.0901983

80 Karamitros D, Kotantaki P, Lygerou Z, Veiga-Fernandes H, Pachnis V, Kioussis D and Taraviras S: Life without geminin. Cell Cycle 9: 3181-3185, 2010. PMID: 20697201. DOI: 10.4161/cc.9.16.12554

81 Karamitros D, Kotantaki P, Lygerou Z, Kioussis D and Taraviras S: T cell proliferation and homeostasis: an emerging role for the cell cycle inhibitor geminin. Crit Rev Immunol 31: 209-331, 2011. PMID: 21740351.

82 Spella M, Kyrousi C, Kritikou E, Stathopoulou A, Guillemot F, Kioussis D, Pachnis V, Lygerou Z and Taraviras S: Geminin regulates cortical progenitor proliferation and differentiation. Stem Cells 29: 1269-1282, 2011. PMID: 21681860. DOI: 10.1002/stem.678

83 Hara K, Nakayama KI and Nakayama K: Geminin is essential for the development of preimplantation mouse embryos. Genes Cells 11: 1281-1293, 2006. PMID: 17054725. DOI: 10.1111/j.1365-2443.2006.01019.x

84 Garzon R, Pichiork F, Palumbo T, Visentini M, Aqeilan R, Cimmino A, Wang H, Sun H, Volinia S, Alder H, Calin GA, Liu CG, Andreff M and Croce CM: MicroRNA gene expression during retinoic acid-induced differentiation of human acute promyelocytic leukemia. Oncogene 26: 4148-4157, 2007. PMID: 17260024. DOI: 10.1038/sj.onc.1211086

85 Zhang J, Gao Y, Yu M, Wu H, Ai Z, Wu Y, Liu H, Du J, Guo Z and Zhang Y: Retinoic acid induces embryonic stem cell differentiation by altering both encoding RNA and microRNA expression. PLoS One 10: e0132566, 2015. PMID: 26162091. DOI: 10.1371/journal.pone.0132566

86 Beveridge NJ, Tooney PA, Carroll AP, Tran N and Cairns MJ: Down-regulation of miR-17 family expression in response to retinoic acid induced neuronal differentiation. Cell Signal 21: 1837-1845, 2009. PMID: 19666108. DOI: 10.1016/j.cellsig.2009.07.019

87 Meseguer S, Mudduluru G, Escamilla JM, Allgayer H and Barettoni D: MicroRNAs-10a and -10b contribute to retinoic acid-induced differentiation of neuroblastoma cells and target the alternative splicing regulatory factor SFRS1 (SF2/ASF). J Biol Chem 286: 4150-4164, 2011. PMID: 21188188. DOI: 10.1074/jbc.M110.167817

88 Zhang L, Cai M, Gong Z, Zhang B, Li Y, Guan L, Hou X, Li Q, Liu G, Xue Z, Yang MH, Ye J, Chin YE and You H: Geminin facilitates FoxO3 deacetylation to promote breast cancer cell metastasis. J Clin Invest 127: 2159-2175, 2017. PMID: 28436938. DOI: 10.1172/JCI90077

89 Vlachakis D, Champeris Tsaniras S, Tsiiliki G, Megalooikonomou V and Kossida S: Molecular modelling study of the 3D structure of the biglycan core protein, using homology modelling techniques. J Mol Biochem 2: 51-58, 2013.

90 Vlachakis D, Champeris Tsaniras S, Tsiiliki G, Megalooikonomou V and Kossida S: 3D structural analysis of proteins using electrostatic surfaces based on image segmentation. J Mol Biochem 3: 27-33, 2014. PMID: 27525250.

91 Vlachakis D, Champeris Tsaniras S, Ioannidou K, Papageorgiou L, Baumann M and Kossida S: A series of Notch3 mutations in CADASIL; insights from 3D molecular modelling and evolutionary analyses. J Mol Biochem 3: 97-105, 2014.

92 Kostaropoulos T, Papageorgiou L, Champeris Tsaniras S, Vlachakis D and Elipoulo E: Carcinogenic pesticide control via hijacking endosymbiosis; The paradigm of DSB-A from Wolbachia pipiensis for the management of Otiorhynchus singularis. In Vivo 32: 1051-1062, 2018. PMID: 30150426. DOI: 10.21873/invivo.11346

93 Kuhn DE, Martin MM, Feldman DS, Terry AV, Nuovo GJ and Ellon TS: Experimental validation of miRNA targets. Methods 44: 47-54, 2008. PMID: 18158132. DOI: 10.1016/j.ymeth.2007.09.005

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