Supplemental Table and Figures

Selective Killing Of Malignant Cancer Cells By Suppression Of Geminin Activity

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SUPPLEMENTAL TABLE

Table SI. Human cell lines used in this study

Human Cell Line	Source	Derivation	Morphology
CCD-841 CoN	CRL-1790	normal colon from human fetus (21 weeks gestation)	adherent epithelial
FHC	CRL-1831	normal colon from human fetus (13 weeks gestation)	adherent epithelial
HCT-116	CCL-247	colorectal carcinoma	adherent epithelial
SW480	CCL-228	colorectal adenocarcinoma	adherent epithelial
COLO 320DM	CCL-220	colorectal adenocarcinoma	Adherent & suspension Rounded & retractile
DLD-1	CCL-221	colorectal adenocarcinoma	adherent epithelial
AG11132	NIACR*	normal breast tissue from 16 year old girl, fail to divide after ~20–25 passages	adherent epithelial
AG11134	NIACR*	normal breast tissue from 28 year old woman, fail to divide after ~20–25 passages	adherent epithelial
MCF-10A	CRL-10317	non-tumorigenic mammary gland	adherent epithelial
MCF7	HTB-22	mammary gland adenocarcinoma	adherent epithelial
WI-38	CCL-75	normal lung fibroblasts from human fetus (3 months gestation)	adherent fibroblast
H1299	CRL-5803	non-small lung carcinoma (p53-)	adherent epithelial
293T	CRL-11268	normal embryonic kidney, later immortalized with SV40 T-ag	adherent epithelial
786-O	CRL-1932	kidney renal cell adenocarcinoma	adherent epithelial
hFOB 1.19	CRL-11372	normal osteoblast, later immortalized with SV40 T-ag	adherent
U-2 OS	HTB-96	osteosarcoma	adherent epithelial

U-87 MG	HTB-14	glioblastoma	adherent epithelial
T98G	CRL-1690	glioblastoma	adherent fibroblast
D-1		normal dermal fibroblast	adherent
K-1		normal epidermal keratinocytes	adherent
WM-266-4	CRL-1676	metastatic site of malignant melanoma	adherent epithelial
A375	CRL-1619	metastatic site of malignant melanoma	adherent epithelial
HeLa	CCL-2	adenocarcinoma of cervix	adherent epithelial

* National Institute of Aging Cell Repository, Coriell Institute for Medical Research. All other cells were obtained from the American Type Culture Collection.

SUPPLEMENTAL FIGURES

Figures S1 - S5. Depletion of geminin in cancer and normal cells. The indicated cells were transfected with siRNA directed against either firefly luciferase (siGL2) or human geminin (siGem). At 48 hours post-transfection, cells were harvested and stained either with propidium iodide to quantify their DNA content by fluorescence activated flow cytometry (FACS) analysis, or with DAPI to visualize their nuclei by fluorescence microscope. The percentage of cells with greater than 4N DNA content is indicated in the FACS profiles. The percentage of cells with giant nuclei (diameter greater than twice that of nuclei in siGL2 treated cells) is indicated in the fluorescence microscopic images. Geminin, GAPDH and actin proteins were detected by Western immuno-blotting of samples in the same gel.

Figure S6. Phase contrast images of HCT116 cells 4 days after transfection with either siGem or siGL2. Giant cells that have rounded up and detached from the dish are indicated by red arrows, and those undergoing apoptosis (i.e. blebbing) are indicated by blue arrows. Both images were taken at the same magnification.

Figure S7. Depletion of geminin by three different siRNAs against geminin leads to DNA re-replication in HCT116 cells but not in HeLa and D-1 cells. HCT116 , HeLa or D-1 cells were transfected with three different siRNAs against geminin (siGem, siGem2 and siGem3) as described in experimental procedures. At 48 hours post-transfection, cells were harvested for FACS analysis or western immno-blotting of samples in the same gel.

Figure S8. Cell sensitivity to siGem was not related to cellular levels of geminin protein. (A) Asynchronously proliferating populations of the indicated cells were subjected to Western immuno-blotting analysis for the indicated proteins. N, normal cells. C, cancer cells. (B) FACS analysis to determine the fraction of proliferating cells (S+G2+M phases) as shown in (A).

REFERENCES

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