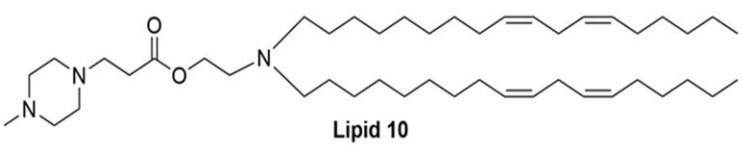
Cancer therapy: Gene silencing of cytoskeleton-associated protein 5 in genetically unstable cancer cells

April 19 2023, by Thamarasee Jeewandara

A



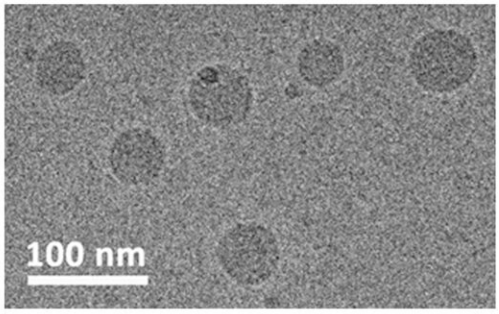
B

Component	Molar ratio
Ionizable lipid	50%
DSPC	10%
Cholesterol	38.5%
DMG-PEG	1.5%

C

	siControl-LNP	si*CKAP5*-LNP
Average size (nm)	73 ± 0.9	76 ± 0.7
Mean number (nm)	57 ± 1.2	61 ± 1.8
PDI	0.055 ± 0.004	0.056 ± 0.004
Zeta	–3.5 ± 0.13	–5.07 ± 1.09
Encapsulation	96% ± 0.04	95% ± 0.02

D



E

Physicochemical properties of siRNA-encapsulated LNPs and their endocytosis. (A) Structure of the ionizable lipid 10 used for LNP preparation. (B) Composition of the particles. (C) Average size (in nm), zeta potential (in mV), PDI (polydispersity index), and % encapsulation efficiency of the particles. Data are represented as mean ± SEM from three batches of formulations (n = 3). (D) Cryo-EM image of lipid nano particles. (E) Confocal microscopic images of

Cy5siRNA-labeled LNP internalization in SKOV3, NAR, and A549 cells. Images are captured at 63×. Cy5 is labeled as magenta, and the cell membrane is stained with rabbit anti-human epidermal growth factor receptor and goat anti rabbit488 antibody. Scale bars, 10 μm. Credit: *Science Advances* (2023). DOI: 10.1126/sciadv.ade4800

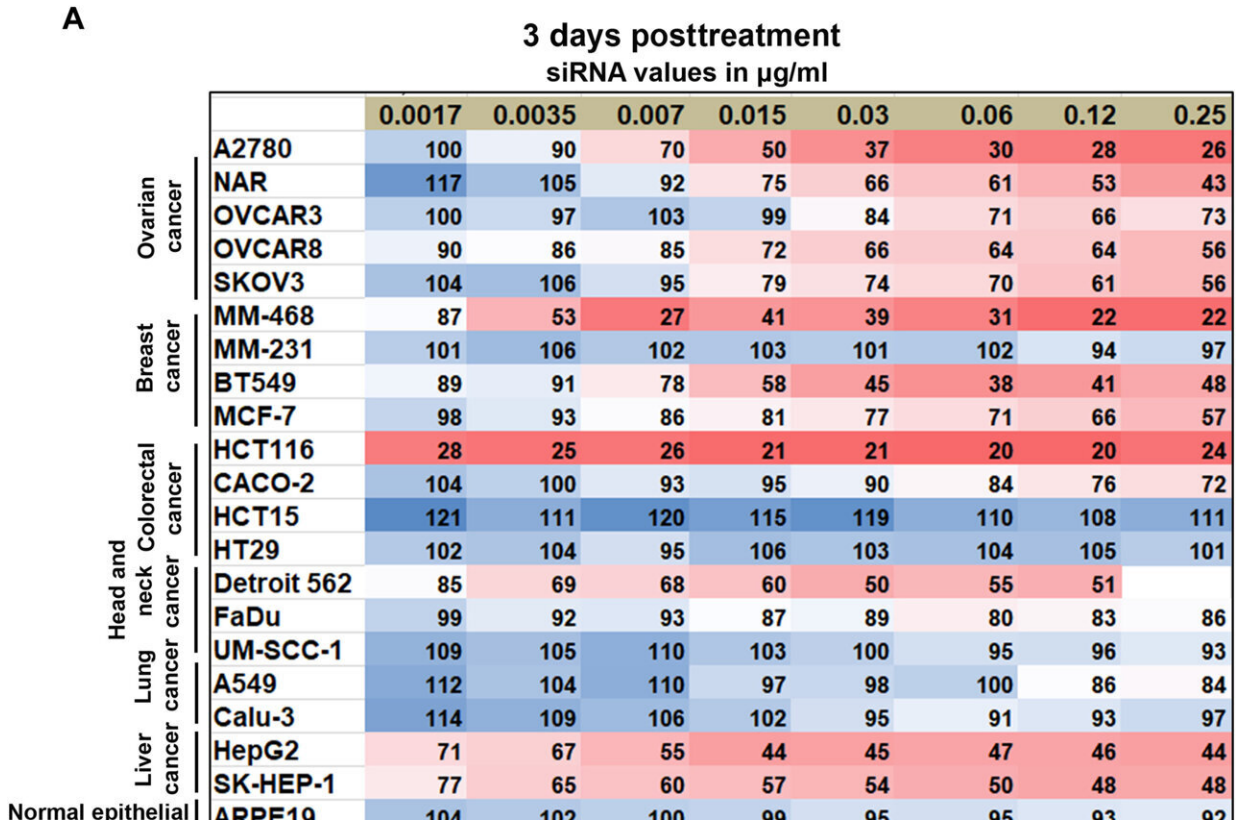
The therapeutic potential of microtubule-associated protein targets for cancer therapy is a largely unexplored research area due to a lack of target-specific agents. Sushmita Chatterjee and a research team in nanomedicine, materials science, nanotechnology and biology at the Tel Aviv University in Israel studied the therapeutic capacity of cytoskeleton-associated protein 5, hitherto abbreviated as CKAP5. The protein is associated with microtubules, and Chatterjee and colleagues silenced the gene via short interfering RNA (siRNA) a molecular biological mechanism to study genes, targeting the CKAP5 encapsulated in lipid nanoparticles for in vivo delivery.

The researchers screened 20 solid cancer cell lines relative to gene silencing to identify a highly responsive chemo-resistant ovarian cancer cell line that underwent significant depletion in mitotic spindle-dynamics for effective experimental cancer treatment. The team showed the therapeutic potential in an ovarian cancer model with an 80% survival rate of silenced-CKAP5 lipid nanoparticle-treated animals. The study is published in *Science Advances*.

The outcomes highlighted the importance of the gene of interest as a therapeutic target to investigate genetically unstable ovarian cancers to further elucidate its mechanisms of action.

New strategies to treat ovarian cancer

Ovarian cancer is a frequent cause of mortality and accounts for about 4.7% cancer mortality among women. The primary treatment procedure includes maximal surgical resection of the tumor, followed by neoadjuvant chemotherapy with a drug combination. While 60%–80% of patients respond to chemotherapy first, about 80%–85% develop chemo-resistance. As a result, there is a constant search for new therapies to treat ovarian cancer. Scientists have explored several drugs targeting specific receptors although such efforts did not yield effective results for patients with ovarian cancer.



		0.0017	0.0035	0.007	0.015	0.03	0.06	0.12	0.25
Ovarian cancer	A2780	100	90	70	50	37	30	28	26
	NAR	117	105	92	75	66	61	53	43
	OVCAR3	100	97	103	99	84	71	66	73
	OVCAR8	90	86	85	72	66	64	64	56
	SKOV3	104	106	95	79	74	70	61	56
Breast cancer	MM-468	87	53	27	41	39	31	22	22
	MM-231	101	106	102	103	101	102	94	97
	BT549	89	91	78	58	45	38	41	48
	MCF-7	98	93	86	81	77	71	66	57
Head and neck Colorectal cancer	HCT116	28	25	26	21	21	20	20	24
	CACO-2	104	100	93	95	90	84	76	72
	HCT15	121	111	120	115	119	110	108	111
	HT29	102	104	95	106	103	104	105	101
cancer	Detroit 562	85	69	68	60	50	55	51	
	FaDu	99	92	93	87	89	80	83	86
Lung cancer	UM-SCC-1	109	105	110	103	100	95	96	93
	A549	112	104	110	97	98	100	86	84
	Calu-3	114	109	106	102	95	91	93	97
Liver cancer	HepG2	71	67	55	44	45	47	46	44
	SK-HEP-1	77	65	60	57	54	50	48	48
Normal epithelial	ARPE19	104	102	100	99	95	95	93	92

Dose-dependent response of CKAP5 knockdown across various cancer cell lines. (A) Cell viability at 72 hours and (B) 6 days post-siCKAP5 treatment as measured by methoxynitrosulfophenyl-tetrazolium carboxanilide (XTT). Cells were treated with the indicated concentration of siCKAP5-LNP and compared with an equivalent concentration of siControl LNP. XTT assay was performed at

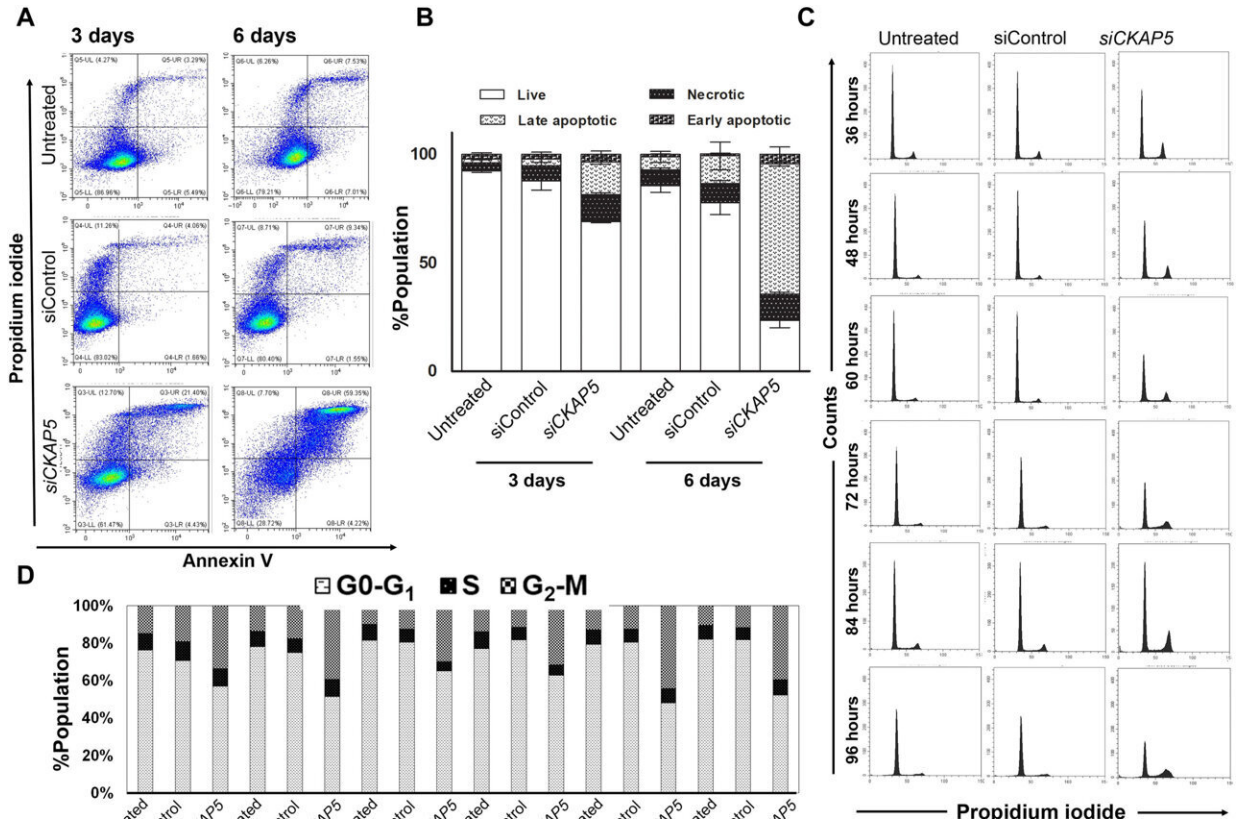
the indicated time points. Values represent average % cell viability compared to cells treated with an equivalent concentration of siControl LNP. Each experiment was carried out at least three times with three technical repeats each time. Cells that did not show any effect of CKAP5 knockdown are highlighted with red boxes. Credit: *Science Advances* (2023). DOI: 10.1126/sciadv.ade4800

Many chemotherapeutic agents explore defects in the cell cycle machinery of cancer cells to halt the cycle through mitosis inhibition. However, existing mitosis-targeting chemotherapeutic agents do not discriminate between healthy and malignant cell lines, resulting in severe side-effects. The challenge therefore is to identify molecular targets associated with mitosis of cancer cells.

Microtubule-associated proteins (MAPs)

Cell mitosis is an attractive target to effectively treat a variety of cancer forms, where microtubule-associated proteins (MAPs) assist cells to maintain the stability of cell dynamics. One such protein is the cytoskeleton-associated protein 5 (CKAP5), widely expressed in a variety of cells to regulate the dynamics of microtubules in human cells.

In this work, Chatterjee and the team screened the effect of CKAP5 silencing in solid cancer cell lines and in normal non-cancer epithelial cell lines as a negative control. While cells with high genetic instability were selectively susceptible to CKAP5 depletion, a chemo-resistant ovarian cancer cell line showed higher sensitivity to cellular intervention, indicating the gene to be a promising therapeutic target in genetically unstable ovarian cancer cell lines.



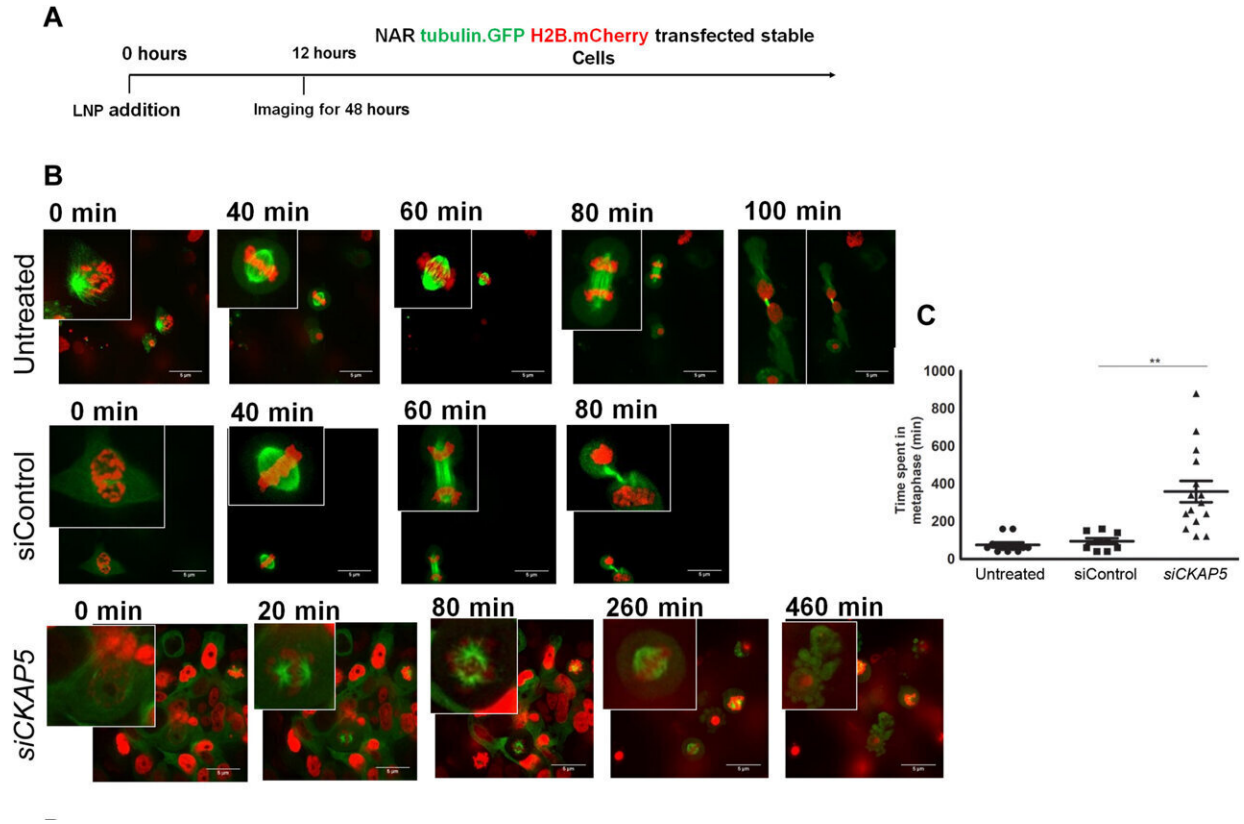
Mechanism of CKAP5 silencing–mediated cell death in NAR cells. In all the experiments, cells were treated with siControl or siCKAP5-LNPs (0.25 μg/ml). (A) Dot plot showing apoptotic and necrotic cells in control and treated cells. Cells were harvested at indicated time points and were subjected to flow cytometry after PI and annexin V–APC staining. (B) Quantitative measurement of the apoptotic cell death. Data represent average % population ± SEM of three representative experiments (n = 2) and analyzed by unpaired t test. (C) Cell cycle analysis over different time periods post-CKAP5 knockdown. Cells were harvested at indicated time points and stained with PI after ethanol fixation. (D) Quantitative measurement of the % cell population in different phases of cell cycle post-CKAP5 knockdown (E) Confocal microscopic images of spindle formation in control and CKAP5 down-regulated cells after 48 hours of treatment. Images were captured at 63× with 7× zoom. Scale bars, 10 μm. (F) Quantitative analysis of the confocal microscopy images to determine the average number of bipolar spindle formation in control versus CKAP5 down-regulated cells. Graph shows % cells with indicated spindles in control and treated cells. Data are represented as average ± SEM of 50 events and analyzed

by unpaired t test. ***P Science Advances (2023). DOI: 10.1126/sciadv.ade4800

High sensitivity to silencing RNA-mediated CKAP5 downregulation in cancer cell lines

The expression of CKAP5 remains to be well documented in a variety of cancer cells. The researchers first studied the expression of this gene at the transcript level in a panel of solid cancer cell lines, including ovarian, colorectal, lung, and head-neck, while examining its expression in non-cancer cell lines as a negative control. The team used SiRNA-encapsulated lipid nanoparticles, formed via microfluidic mixing, to create uniformly sized structures and examined the outcomes via cryo-electron microscopy.

Of the cell lines tested, they noted eight that were resistant to CKAP5 silencing, including normal or healthy cell lines, which meant that normal cells did not respond toward CKAP5 genetic downregulation. All ovarian cancer cell lines were sensitive to gene silencing; the team therefore exclusively explored this lineage for further studies. While the team downregulated the gene of interest in all cell lines, only specific cancer cells with high genetic instability were vulnerable to CKAP5 depletion.



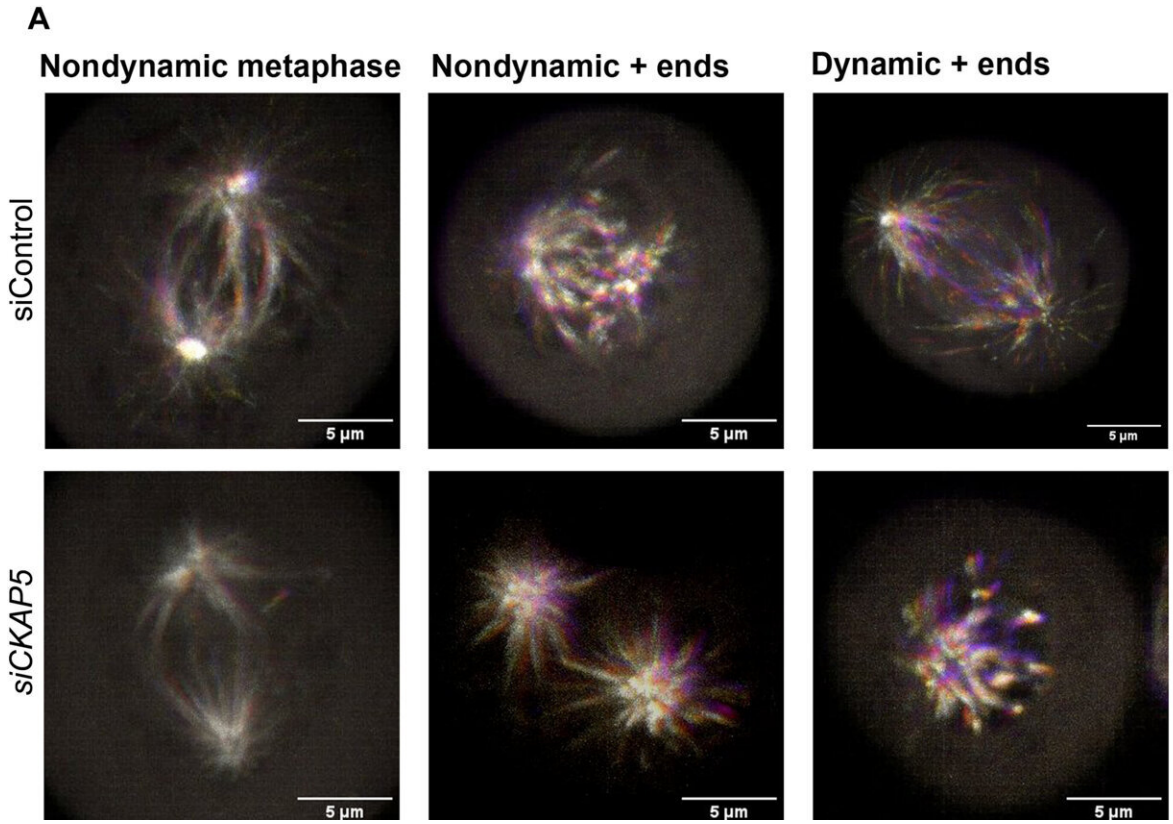
Live cell tracking of CKAP5-silenced NAR cells. Cells were labeled with tubulin.GFP and histone H2B.mCherry to follow the mitotic spindle and nucleus respectively. (A) Schematic representation of the experiment performed indicating the timelines of LNP addition and live-cell imaging. (B) Representative images to follow cell cycle in NAR cells treated with siControl or siCKAP5-LNP. NAR cells labeled with Tubulin.GFP and histone H2B.mCherry were treated with siControl or siCKAP5-LNPs (0.25 μg/ml). Twelve-hour posttreatment live-cell imaging was performed by a spinning disk confocal microscope. Images were captured every 15 min for 48 hours. Scale bars, 5 μm. (C) Average time spent in mitosis in different groups as observed with a spinning disk microscope. Data are represented as average time ± SEM from 20 cell cycle events and analyzed by unpaired t test. **P = 0.0019. (D) Cell fate with respect to time in control and treatment groups. Each bar represents a cell and its fate through different phases of the cell cycle. Credit: *Science Advances* (2023). DOI: 10.1126/sciadv.ade4800

Mechanisms of CKAP5 down-regulation: Cell cycle arrest and spindle defects in ovarian cancer cells

The cancer biologists examined the mechanisms of CKAP5 down-regulation-mediated cell death by developing an apoptotic assay, using propidium iodide-annexin V staining. They noted cell apoptosis (cell death) after transfecting silenced genes into cell lines of interest by using lipid carriers. Spindle damage in response to CKAP5 depletion showed a significant increase in cells arrested in metaphase, compared to control cell lines and untreated cells. The process involved multicentric spindle formation, cell cycle inhibition, and apoptosis, accompanied with spindle checkpoint gene upregulation.

The team studied cell cycle inhibition in two other ovarian cancer cell lines, to show similarities in their behavior to indicate the inhibition of cell viability. When they compared these results in noncancerous normal epithelial cell lines, they remained unaffected in viability and cell cycle state. While all sensitive cancer cell lines underwent increased apoptotic cell death in response to the CKAP5 gene knockdown, there were non-responsive cancer cell lines too that did not undergo apoptotic cell death.

The researchers examined the mechanisms of cell apoptosis in gene-silenced cell lines, to understand the fate of cells. Since the silenced gene was transferred via lipid carriers, they also studied the lipid nanoparticle biodistribution localized in the liver, spleen, and tumor tissues, via fluorescence-activated cell sorting methods.

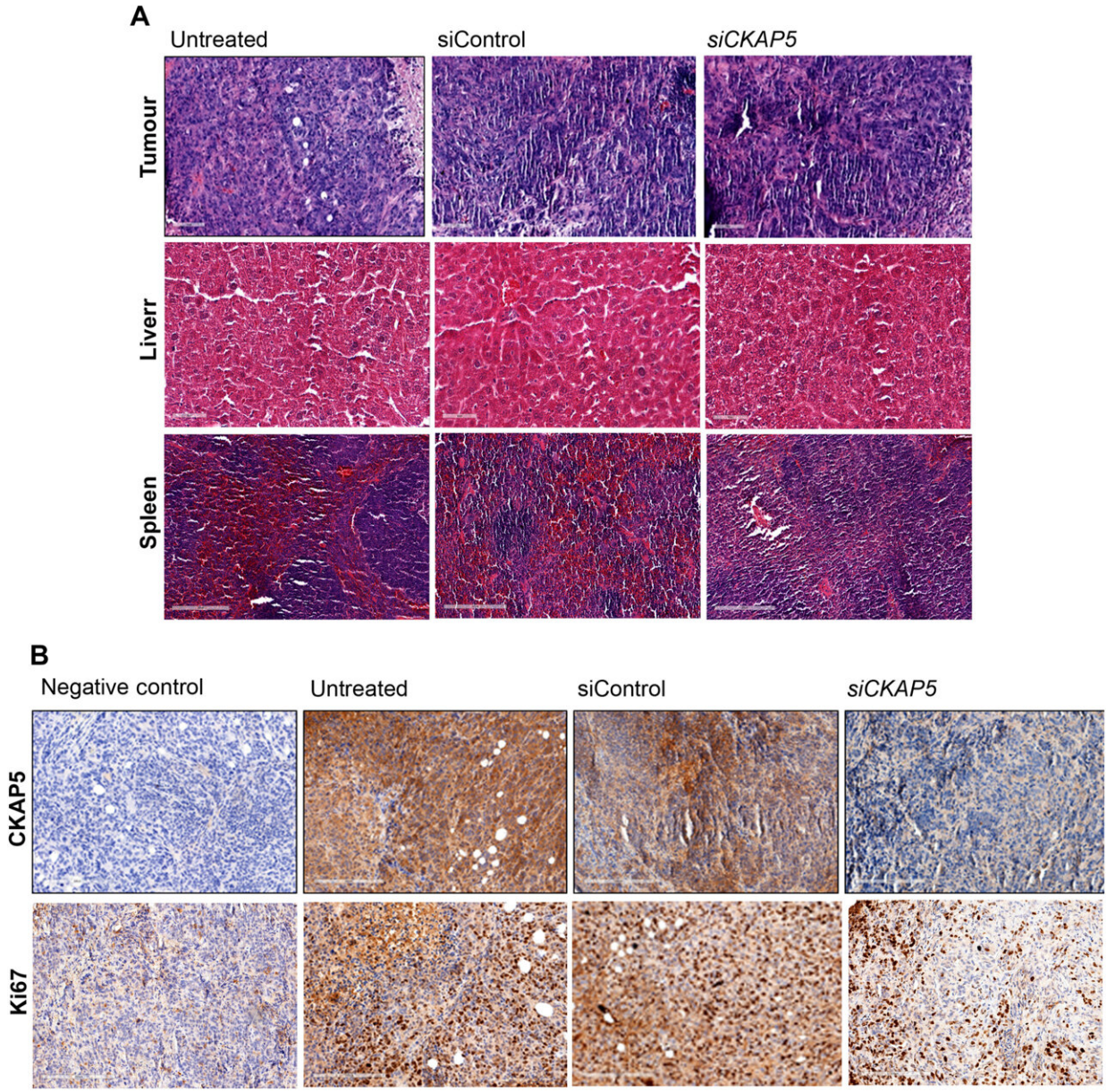


CKAP5 silencing–mediated effects on microtubule dynamics and localization of NAR cells during mitosis. (A) Representative phenotypes of EB3 localization in control and CKAP5-silenced groups. NAR cells were transiently transfected with EB3.eGFP plasmid to label the microtubule + ends. After 12 hours of transfection, cells were further transfected with siControl/siCKAP5 LNPs, and live-cell imaging was performed after 30 hours of siRNA-mediated silencing. Live cell imaging was performed by a superresolution spinning disk microscope. Images were captured at 100×. Scale bars, 5 µm. Images were captured every second for a period of 90 s. The images represent superimposition images of 91 frames with each frame marked in a specific shade as shown in the time frame scale. Nondynamic + ends superimpose on each other in all 90 frames due to their static nature and represent as white due to superimposition, whereas dynamic + ends do not superimpose in all the 90 frames due to their dynamic behavior and thus appear as a rainbow color. Thus, higher dynamics of tubulin result in a wider range of colors in these representative superimposed images. (B) Quantitative representation of the microtubule (MT) growth speed in control and CKAP5-silenced group. The live-cell kinetic data obtained from a spinning

disk microscope was subjected to Utrack analysis using MATLAB software. **P = 0.0097. (C) Quantitative representation of the MT growth lifetime in control- and CKAP5-silenced group as measured by MATLAB analysis. (D) Quantitative representation of the MT growth length in control and CKAP5-silenced group as measured by MATLAB analysis. For all the graphical analysis in (B) to (D), the data are represented as mean ± SEM from 10 mitotic events and statistically analyzed by an unpaired t test. Credit: *Science Advances* (2023). DOI: 10.1126/sciadv.ade4800

Xenografted tumor growth and increased survival in gene-knockout animal models

The team tested the effects of therapeutic gene silencing in animal models after implanting the genetically modified cell lines into mice to monitor tumor growth. After eight days of implantation, they randomly grouped the mice to treated, control and untreated groups. After administering several doses, they studied the dynamics of tumor growth via fluorescence and luminescence imaging in vivo. After sacrificing the animal models after treatment, they collected the tumor tissues to test in vivo silencing efficiency, and assessed the tissue anatomy. As expected, the animals delivered with the silenced gene yielded substantially reduced genetic expression, when compared to control groups to suggest experimental success.



Histological investigation of various tissues extracted from siControl and siCKAP5-treated mice. (A) H&E staining of liver, spleen, and xenografted tumor tissues isolated from untreated, siControl, and siCKAP5-LNP–treated mice. Scale bars in tumor and liver tissue represent 100 μm. Scale bars in spleen tissue represent 200 μm. (B) Immunohistochemistry of ovarian cancer peritoneal tumor tissue shows CKAP5 and Ki67 expression in untreated, siControl, and siCKAP5-LNP–treated group. Scale bars, 200 μm. Credit: *Science Advances* (2023). DOI: 10.1126/sciadv.ade4800

Outlook

For decades, scientists have targeted the cell cycle to explore cancer therapies to overcome the chromosomal abnormalities and genetic defects associated with cancer cells. At present, tubulin targeting agents are at the forefront of antimitotic agents. However, researchers urgently seek to explore new mitotic targeting agents. In this way, Sushmita Chatterjee and colleagues showed the cytoskeleton-associated protein 5 (CKAP5) gene to play an important role in mitotic spindle assembly by affecting tubulin function. Its inhibition can play a key role to treat genetically unstable ovarian cancer cells.

The study outcomes highlight the vulnerability of cancer cells to CKAP5. Since ovarian cancer is diagnosed at late stages, it will be productive to study the possibility of silencing CKAP5 in late-stage ovarian cancer metastasis, with implications to treat other cancers that metastasize and localize to the intraperitoneal cavity, including pancreatic, liver, and colorectal cancers.

More information: Sushmita Chatterjee et al, Therapeutic gene silencing of CKAP5 leads to lethality in genetically unstable cancer cells, *Science Advances* (2023). DOI: 10.1126/sciadv.ade4800

Deborah K. Armstrong et al, Intraperitoneal Cisplatin and Paclitaxel in Ovarian Cancer, *New England Journal of Medicine* (2006). DOI: 10.1056/NEJMoa052985

associated.html