Weakened RNA interference reduces survival in ovarian cancer

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Levels of two proteins in a woman's ovarian cancer are strongly associated with her likelihood of survival, a research team led by scientists at The University of Texas M. D. Anderson Cancer Center reports in the Dec. 18 issue of the *New England Journal of Medicine.*

The study shows that women with high levels of Dicer and Drosha, two proteins that are vital to a cell's gene-silencing machinery, had a median survival of 11 years. For those with low levels of either or both proteins, median survival was 2.66 years.

"Dicer and Drosha are crucial for two types of RNA interference, which cells use to shut down genes. We've found that when this machinery is disrupted, patient outcomes are poor," said senior author Anil Sood, M.D., professor in the departments of Gynecologic Oncology and Cancer Biology at M. D. Anderson. The study is the largest and most comprehensive demonstration of the connection between RNA interference and cancer.

The researchers also analyzed gene expression data in groups of lung and breast cancer patients and found similar associations with patient survival.

"Very consistently, we found that low levels of Dicer in particular are predictive of poor outcomes," Sood said. Molecular details of the raised risk for patients remain to be discovered, but it is likely that low levels of Dicer and Drosha permit some genes to continue functioning when

they should be silenced.

"RNA interference has only been known for about a decade. The components of the machinery, what it does in cancer, and how it affects outcomes and therapy are not fully known," Sood said.

Potential clinical applications include using levels of the proteins as prognostic indicators to guide treatment decisions and eventually to exploit RNA interference to attack tumors, Sood said.

Interfering with gene expression

The team measured expression levels of Dicer and Drosha in 111 invasive ovarian cancer tumors and then compared the results to the patients' clinical outcomes. The initial findings were supported by a second analysis of gene expression in a different group of 132 ovarian cancer patients.

Analysis of 91 patients with lung cancer and 129 breast cancer patients reached similar conclusions, however, only Dicer levels were found to affect survival.

Drosha and Dicer are involved in the production of short interfering RNA (siRNA) and micro interfering RNA (miRNA). Genes express messenger RNA to tell a cell's protein-making machinery what protein to make. SiRNA and miRNA work by either cleaving the target messenger RNA or preventing protein production.

Drosha prepares pre-miRNA in the nucleus so it can be ejected into the cell's cytoplasm, where Dicer chops it into workable pieces of miRNA. Separately in the cytoplasm, Dicer cuts double-stranded RNA into bits of siRNA. Both miRNA and siRNA must go through Dicer to function naturally in the cell. Therapeutic pre-processed siRNA does not require

Dicer and can be introduced into the cells as potential treatment, Sood noted.

Statistical analysis of five risk factors for ovarian cancer showed that only low Dicer levels, high-grade tumors and poor response to chemotherapy are independent predictors of survival. "When we find a new prognostic factor for cancer, we conduct a multivariate analysis to make sure that it's not associated with known factors, such as tumor grade. In this case, low Dicer levels were completely separate from traditional predictive factors," Sood said.

A genetic analysis of the Dicer and Drosha genes turned up mutations in both, but none that were associated with high or low levels of the proteins.

siRNA's potential for treatment

Sood and colleague Gabriel Lopez-Berestein, M.D., professor in M. D. Anderson's Department of Experimental Therapeutics, are developing cancer drugs that deliver a siRNA via fatty nanoparticles to silence a specific cancer-causing gene.

"We've found that another type of RNA, short hairpin RNA (shRNA) silences genes in a stable manner rather than the transient effects attributed to siRNA," said study senior co-author Menashe Bar-Eli, Ph.D., professor of Cancer Biology. However, animal models showed that these longer shRNA fragments could not silence genes in some cells. The authors found that about half of ovarian cancer cells either had low levels of Dicer and Drosha or lacked one or both proteins altogether.

A functional test of Dicer and Drosha for the project showed that shRNA does not work well with low levels of Dicer. When Dicer is low, siRNA still works. "This suggests that for therapeutic purposes, siRNA

might be the better option as we develop new treatments based on interfering RNA," said co-author Robert Coleman, M.D., professor of gynecologic oncology.

Source: University of Texas M. D. Anderson Cancer Center