

## Path involving chromosome "caps" may set malignancy in motion

Research has repeatedly shown that a high rate of spontaneous mutations occurs in the genetic makeup of cancerous tumors, but the jury is still out on the cause of this genetic instability and the contributions, if any, of these aberrations to the malignancy. New studies by Paul Yaswen, Ph.D., of the Lawrence Berkeley National Laboratory, suggest that a combination of factors, including the shortening of the caps that sit on the ends of chromosomes, may instigate the process that leads to cancer.

Dr. Yaswen is using surgically-discarded, human mammary epithelial cells grown in culture (in artificial conditions in the lab). He is especially interested in the growth controls that are typical of normal cells, but are absent in malignant cells. Normal cells grow for a while in culture, but then stop in a phase called senescence, which appears to be mediated by a tumor suppressor called p16, he said.

"Interestingly, a minority of cells in culture manage to downregulate (shut down) the suppressor and continue to grow. These cells encounter another senescence arrest called agonescence, and this appears to be due to critical shortening of structures, called telomeres, at the ends of the chromosomes."

Under normal conditions, the telomere end caps protect the chromosomes and ensure that the genetic information (DNA) they contain is passed on properly. Without the caps, the genetic information would be susceptible to damage. This might include the loss of some DNA, the fusing of chromosomes together, or myriad other mutations. Every time cells divide, their telomeres become a little shorter. When they become too short and the genetic information contained in the chromosomes is in jeopardy, the cells stop growing or die.

Unlike normal cells, those in breast tumor tissue bypass these controls and continue growing and dividing indefinitely, Dr. Yaswen said. Results in his lab and others show that a main culprit is an enzyme called telomerase. This enzyme maintains the telomeres. In humans, telomerase is active in fetal tissue, and in adult sperm and eggs, but not in other normal, body cells. Tumor cells, however, are able to activate telomerase, boost the length of their telomeres, and become immortal, he explained. "In normal cells, we think that telomerase is stringently repressed as a mechanism for suppressing carcinogenesis, and it is stringently repressed by more than one independent mechanism."

How, then, do the tumor cells switch on telomerase production? To find out, Dr. Yaswen collaborated with Dr. Martha Stampfer, also at the Berkeley Lab, to examine cell cultures previously treated with benzopyrene, a known carcinogen found in such sources as cigarette smoke. He said, "We did not detect the activity of the telomerase immediately after the carcinogen exposure, but only many generations following the exposure. We now think that the benzopyrene can cause errors in a pathway that's responsible for repressing telomerase, but that other errors have to accumulate before the telomerase can actually turn back on."

He believes that these other errors might come during the period when the telomeres are critically short. This is an especially vulnerable time for the chromosomes, because the telomeres are no longer able to protect the genes contained in the chromosomes and they are more likely to mutate. Normally, this is not a problem, because the cells with critically short telomeres soon stop growing or die. If mutated cells manage to turn on telomerase, however, they can extend their telomeres and become immortal, a critical step in becoming tumor cells.

Dr. Yaswen's lab took this information and tested a gene, called ZNF217, that has been linked to malignancy. "When we put this ZNF217 gene into cells, the telomerase didn't come on again immediately, so we concluded that the ZNF217 gene is not turning on telomerase activity itself." In this case, he believes that the aberrant overexpression of the gene is reducing the tight control of telomerase, and increasing the survival of cells with shortened and therefore dysfunctional telomeres until a second event occurs that finally turns on the telomerase and subsequently lengthens the telomeres, giving the cells a new lease on life.

In summary, he said, "We think that most spontaneous, human, solid tumors arise from telomerase-negative cells, which acquire their malignant changes during this early period of genomic instability that is associated with telomeric dysfunction. Having acquired these malignant changes, the tumor cells reactivate telomerase in order to survive, and in so doing they may acquire additional features which are common in stem cells." Stem cells, seen in fetal development, are cells that are generalized, or undifferentiated, and have the ability to grow into various types of tissue cells, such as lung, heart or muscle cells. These cells require telomerase, which protects their chromosomes and gives them an extended growth period.

Further investigation into the reason that telomerase repression fails could be important in the fight against breast cancer. He said, "We think augmentation of the processes that monitor and prevent the growth of these cells with the dysfunctional telomeres may be useful for the prevention of immortalization and the prevention of malignancy."

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Summary of Dr. Yaswen's presentation at the November 2005 BCERC Scientific Symposium:  
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