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Vitamin D deficiency contributes to spread of breast cancer in mice, study finds

March 2 2016

Breast tumors in laboratory mice deficient in vitamin D grow faster and are more likely to metastasize than tumors in mice with adequate levels of vitamin D, according to a preliminary study by researchers at the Stanford University School of Medicine.

The research highlights a direct link between circulating vitamin D levels and the expression of a gene called ID1, known to be associated with tumor growth and breast cancer metastasis.

The finding builds upon several previous studies suggesting that low levels of vitamin D not only increase a person's risk of developing breast cancer, but are also correlated with more-aggressive tumors and worse prognoses. Although the research was conducted primarily in mice and on mouse cells, the researchers found in a study of 34 breast cancer patients that levels of circulating vitamin D were inversely correlated with the expression levels of ID1 protein in their tumors, and they confirmed that a vitamin D metabolite directly controls the expression of the ID1 gene in a human breast cancer cell line.

"Although much more research needs to be done, research from our lab and others suggests that people at risk for breast cancer should know their vitamin D levels and take steps to correct any deficiencies," said Brian Feldman, MD, PhD, assistant professor of pediatrics.

Feldman, who is a Bechtel Endowed Faculty Scholar, is the senior author of the study, which will be published online March 2 in *Endocrinology*.

Lead authors of the work are graduate student Jasmaine Williams and postdoctoral scholar Abhishek Aggarwal, PhD.

Confusion about optimal dosage

The researchers emphasize that their findings don't imply that more vitamin D is always better. Correcting a deficiency is very different from taking more than the recommended dosage, which the Institute of Medicine says is 600 international units per day for people age 70 and younger, and 800 IU for older adults. Excess levels, variously estimated to occur at about 4,000 to 10,000 IU per day, have been linked to damage to the kidneys, cardiovascular system and other organs.

Not all medical organizations agree on the optimal amount of vitamin D. The confusion stems in part from the fact that, although it can be ingested via food and nutritional supplements, our bodies can also make vitamin D with the help of ultraviolet rays from the sun. So it's difficult to know exactly how much any individual may need to take as a supplement, and that amount can vary throughout the year. Those who don't get enough sun exposure, or people with darker skin, are more likely than fair-skinned individuals who spend time outdoors each day to be deficient. The use of sunscreen can also affect vitamin D synthesis.

Once ingested or made by the body, vitamin D is converted through a series of steps into its active form, calcitriol. Calcitriol binds to a protein in cells called the vitamin D receptor, which then enters the cell's nucleus to control the expression of a variety of genes, including those involved in calcium absorption and bone health.

A brake on tumor progression?

The link between vitamin D and calcium metabolism is well-known.

More recently, however, researchers have begun to suspect that vitamin D may affect many other important biological processes, including tumor progression. However, it's not clear exactly which step in cancer development the vitamin may affect.

In the new study, Williams and Aggarwal investigated whether vitamin D levels affected the metastatic ability of mouse breast cancer cells implanted into the mammary fat pad of laboratory mice. One group of 10 mice was first fed a diet lacking in the vitamin for 10 weeks; the other 10 received a normal dose in their food.

Mice fed a diet deficient in vitamin D developed palpable tumors an average of seven days sooner than their peers, and after six weeks of growth those tumors were significantly larger in size than those in animals with adequate vitamin D levels.

The researchers then examined two well-characterized lines of mouse tumor cells, 168FARN and 4T1. Prior research has shown that cells from either group form tumors when implanted in laboratory mice, but only 4T1 results in aggressive tumors that spread to other parts of the animal's body.

Vitamin D and ID1 expression

The researchers found that the 4T1 cell line expresses significantly lower levels of the vitamin D receptor protein. When they genetically engineered 168FARN cells to also have lower-than-normal levels of the VDR protein, the cells began to behave much more like the 4T1 cells. They migrated more freely in a laboratory dish and, when injected into 10 mice, they grew aggressively. In six of these mice, the modified cancer cells metastasized to the liver during the course of four weeks. In contrast, none of the tumors in the 10 mice that received unmodified 168FARN cells spread to the liver during the study period.

To identify how vitamin D might be affecting metastasis, the researchers analyzed gene expression in the tumors that developed in mice with varied levels of vitamin D in their diets and in the tumors of mice injected with modified or unmodified 168FARN cells. They found that in cases in which vitamin D was lacking from the diet or in which cells were missing much of the VDR protein, tumor cells expressed more of a gene called ID1, which has been shown to play a role in breast cancer metastasis. Further investigation showed that VDR binds directly to a stretch of DNA near the ID1 gene and suppresses its expression in both mouse and human cells.

Finally, the researchers compared circulating vitamin D levels in 34 breast cancer patients at Stanford with the levels of ID1 in tumor cells that were surgically removed during the course of disease treatment. They found an inverse correlation: Women with lower levels of vitamin D expressed more ID1 in their tumor tissues than did women with higher levels of vitamin D.

"Our study shows that a deficiency in vitamin D levels, or an inability of tumor cells to respond appropriately to the presence of vitamin D, is sufficient to trigger non-metastatic cancer cells to become metastatic," said Feldman. "It's enough to significantly accelerate tumor progression in our mouse model. Further studies are warranted, but this direct association between vitamin D levels and ID1 expression is very interesting to us."

Provided by Stanford University Medical Center