Hens as protein bioreactors

Egg whites—naturally fat-free, low in calories, and high in protein—may soon add therapeutic proteins to their litany of health benefits. Researchers have created genetically modified hens that lay eggs containing high levels of these functional therapeutic proteins in the egg whites, potentially laying the groundwork for the development of a faster and cheaper protein production method.

Therapeutic proteins, such as monoclonal antibodies and enzymes, are useful for treating diseases like cancer and diabetes. The current production methods of choice for these molecules are bacterial fermentation and mammalian cell culture, techniques that are extremely time-consuming and expensive, prompting researchers to seek alternative protein production platforms. One promising approach to this problem is creating transgenic animals that produce the proteins of interest. To date, researchers have produced transgenic goats, sheep, and cattle that secrete therapeutic proteins in their milk. These animals, however, are expensive to house and have long generation times.

Recent advances in avian transgenesis have made it theoretically possible to use transgenic chickens as 'protein bioreactors.' The shorter generation time, lower maintenance costs, and the potential to produce proteins toxic to mammalian cells are among the projected advantages of transgenic chickens over their mammalian barnyard mates.

Now, Helen M. Sang of the Roslin Institute (Midlothian, Scotland) and coworkers report successfully transfecting chicken embryos with a lentiviral vector (*Proc. Natl. Acad. Sci. USA*, 6 February). The vector, equine infectious anemia virus, had been loaded with transgenes encoding miR24 (a miniantibody for treating malignant melanoma) and human IFN-β-1a (hIFNβ1a, an antiviral cytokine) under the control of regulatory sequences that restrict expression to the oviducts, where the egg whites are formed.

Sang and her associates then crossed transgenic cockerels with stock hens and analyzed the offspring for the presence of the transgene. ELISA testing of egg whites from eggs laid by G_1 and G_2 hens confirmed the presence of recombinant protein in all of the eggs assayed and protein levels remained consistent over time. Later, testing of the recombinant hIFNβ1a in a cytopathic effect assay demonstrated that the protein was functional. Moreover, northern blot analysis of various tissue types confirmed that expression of the transgenes was limited to the oviduct—an important consideration since certain therapeutic proteins could be toxic to the chicken if produced throughout the body.

Sang's team will continue to analyze transgene expression levels to determine whether transgene silencing occurs in subsequent generations. The recombinant proteins have yet to be tested in humans, meaning that clinical application of this research is probably still several years away.

Tanja Schub

BLOCKING BONE DESTRUCTION IN BREAST CANCER

Researchers have identified a molecular mechanism by which breast cancer metastases promote bone destruction. Inhibition of this process may help prevent one of the most common—and most agonizing—complications of breast cancer.

Tumors of the breast are the most common form of cancer among women in developed countries. Breast cancer frequently spreads to the bones; indeed, more than half of patients develop osteolytic skeletal lesions. These women endure unrelenting pain and an increased risk of pathological fracture and hypercalcemia.

In a recent paper in *Nature Medicine* (January), Cun-Yu Wang at the University of Michigan (Ann Arbor) and coworkers have identified what purports to be the molecular culprits at the root of the bone resorption associated with breast cancer: nuclear factor-κB (NF-κB) and granulocyte macrophage-colony stimulating factor (GM-CSF). Researchers have long considered NF-κB a dangerous molecule that aids and abets developing tumors in many kinds of cancer. Wang and associates, however, are the first to report a causative connection between NF-κB, GM-CSF, and the bone destruction associated with breast cancer.

Two experiments were essential in leading Wang's team to these conclusions, both of which involve a murine model of osteolytic bone metastasis created by injecting breast cancer cells into the left cardiac ventricles of immunodeficient mice. In the first experiment, cancer cells modified to repress NF-κB were injected into mice; of these, far fewer developed bone lesions as compared to controls, and of those that did develop lesions, they were smaller than those of control mice injected with unmodified tumors.

Next, Wang and colleagues examined the gene expression differences between the two types of cells and determined that some genes, including the one for GM-CSF, were not expressed as much in the cells with NF-κB repression than in unmodified tumor cells. These findings were unexpected considering that GM-CSF is used in some instances as a therapeutic agent to stimulate the recovery and proliferation of the blood cells killed by chemotherapy. Therefore, in a second experiment, Wang and colleagues injected mice with the NF-κB-repressed tumor cells used before, as well as NF-κB-repressed cells that they had modified to artificially increase GM-CSF expression. The former cells caused little or no bone lesions, but the latter caused extensive bone lesions and damage.

The next step for researchers is to turn these findings into practical treatment regimes. It may be possible, for instance, to limit or prevent bone metastasis of breast cancer by blocking NF-κB or GM-CSF in cancer patients. Alternatively, increased GM-CSF might be used to flag patients with a high risk of bone metastasis.

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