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RNA Interference for Therapeutic Treatment of Breast Cancer: How to Tackle the Issue of Multidrug Resistance

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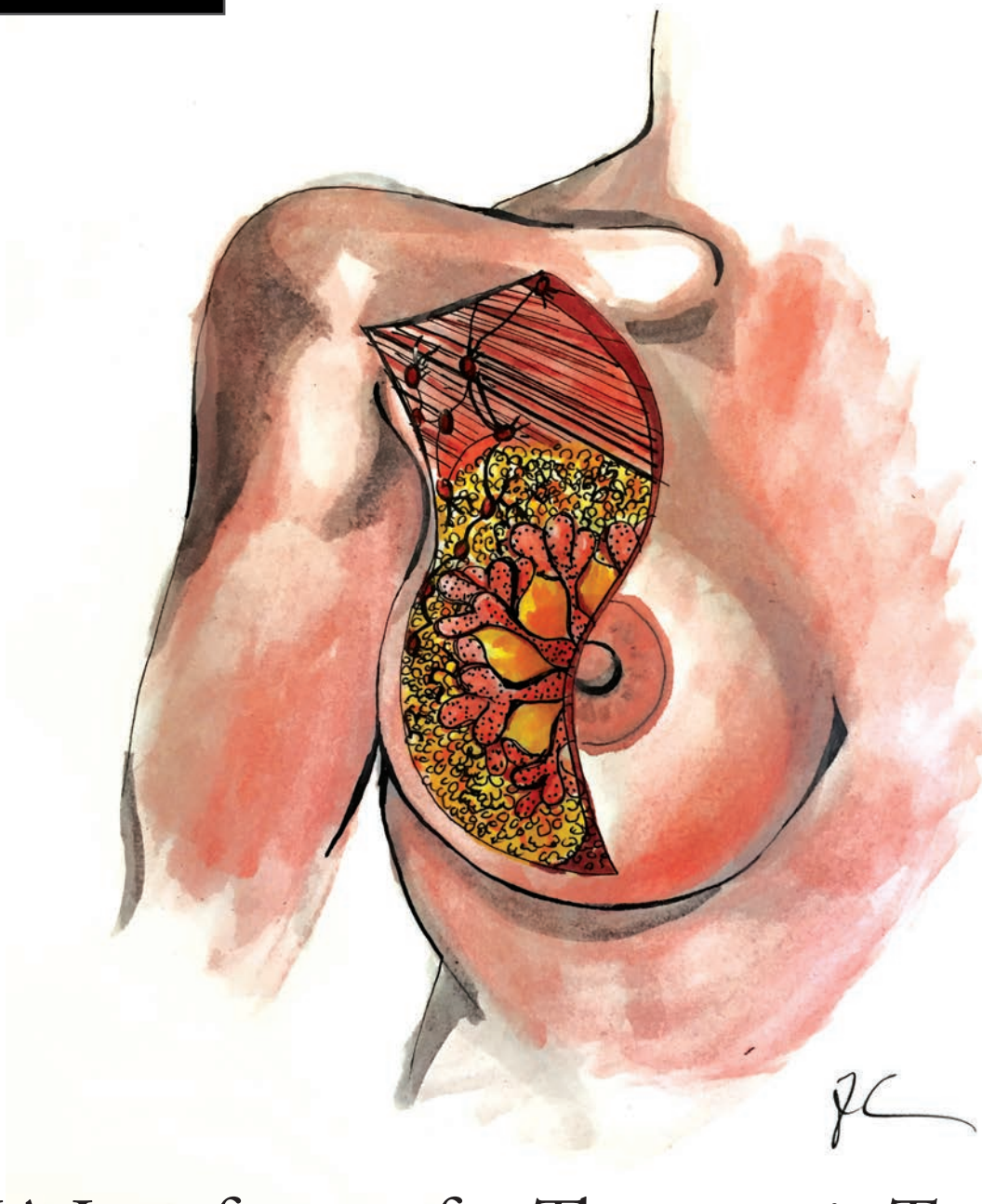

RNA Interference for Therapeutic Treatment of Breast Cancer

How to Tackle the Issue of Multidrug Resistance

Written by Carson McCann
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Science fiction often projects ideas that seem absurd at the time of their release, but as time endures and scientific knowledge expands we find those otherworldly ideas become a typical part of life. Star Trek's Captain Kirk used the equivalent of a cell phone years before its invention. John Brunner's *Stand on Zanzibar* foretold of motor vehicles powered by rechargeable electric fuel cells. Car companies like Tesla, Porsche, and BMW are designing and selling electric sports cars. Further, Aldous Huxley's *Brave New World* depicted a future in which children are genetically modified by changing their developing environments. Despite the shocking thought of genetically-engineered children, modern society is taking steps toward the ability to selectively modify the human genome, and its potential is limitless.

Gene expression control has remained a heated topic of debate due to the ethical issues relating to its far-reaching potential. Research has demonstrated the ability to control gene expression by placing a blockage in the very central dogma of biology. The dogma states that our hereditary information passes from deoxyribonucleic acid (DNA) to ribonucleic acid (RNA) via the process of transcription. The RNA is then transcribed and protein expression leads to phenotypic observation. Therefore, researchers contemplated the idea of blocking the passage of

information from RNA to protein expression. Studies have found that if you design a complementary sequence of RNA for a specific gene and inject it into a cell you can 'erase' its expression via a gene knockout. This process of gene expression control is termed RNA interference (RNAi). The ability to manipulate the expression of genes can reach as superficially as changing hair color or as impactful as treating and preventing deadly diseases. Breast cancer remains a disease of high morbidity and mortality due to the proximity of the targeted tissue to other vital organs. Cancerous cells in the breast can metastasize to regional lymph nodes, bone marrow, the lungs, and the liver. A metastasis in any of these areas is often fatal. RNAi's ability to 'knock down' the expression of an overexpressed gene has been shown to be applicable in combating the dilemma of multidrug resistance, when cancer cells evolve a resistance or immunity to drugs that were once successful. Although RNAi can be used to directly kill cancer cells, its ability to enhance the activities of other drugs is extremely valuable for combination drug therapy.

Before we can understand RNAi's ability to treat cancer patients, it is important to appreciate the pathway by which RNAi works. The mechanism for RNAi is a complex interplay of RNA modifications, enzyme guidance, and variable mRNA regulation. The mechanism starts when a double-stranded RNA (dsRNA) is injected into the cell. When dsRNAs are present in the cytoplasm of a eukaryotic cell, an enzyme called Dicer will recognize and cut the RNA into approximately 22 nucleotide-long strands. The processed product is termed small interfering RNA (siRNA). RNA-induced silencing complex (RISC) then recognizes the siRNA. RISC contains a protein called Argonaute, which binds to the RNA and has endonuclease activity. The RISC-Argonaute complex will splice the siRNA into a single strand, after which it will use the single-stranded siRNA sequence to cleave a specific complementary messenger RNA (mRNA) in the cell. If the siRNA sequence matches the targeted mRNA sequence exactly, RISC will cleave the strands for mRNA degradation. However, if there is only partial complementarity with the siRNA sequence, there will just be translational inhibition. In both scenarios the translation of proteins is blocked for the specific gene. The ability to knock down the expression of a gene has intrigued cancer researchers. Breast cancer is a disease characterized by mutations leading to the overexpression of oncogenes and a subsequent loss of function of tumor suppressor genes. In addition, cancer cells' constant aberrational mutations promote resistance to designed drugs. Researchers have found that RNAi techniques can knock down critical hyperactive oncogenes and induce sensitivity to cancer treatment drugs. Due to the prevalence, mortality, and morbidity of breast cancer in the human population, RNAi could serve as an essential therapy to save lives.

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Resistance to chemotherapeutic drugs is a serious challenge faced in the clinical practice to induce apoptosis in tumor cells. Most anti-tumor drugs induce programmed cell destruction, but cancer cells can develop a resistance to the antineoplastic medicines. This resistance can extend to multiple substances in a process known as multidrug resistance (MDR), which ultimately hinders the effectiveness of chemotherapy. Many forms of MDR come from the overexpression of ATP-binding cassette transporters, which are transmembrane proteins that use ATP to move a substance from one side of the membrane to another. One example of a significant ATP-binding cassette transporter in cancer is the breast cancer resistance protein (BCRP).

BCRP facilitates MDR by pumping toxic substances out of tumor cells. Upon initial chemotherapy treatment, the cancer cells do not express BCRP at a sufficient level to adequately transport the apoptosis-inducing chemicals outside the cell. Most of the cancer cells will die; however, one of the key traits of these cells is their tendency to mutate at a rapid rate. Eventually, a cancer cell will likely adapt in such a way to survive the chemical treatment. Natural selection will isolate those adaptive cancer cells, and as a result, the cancer cells become resistant to the drug. Cancer cells adapted to survival often have increased production of BCRP. Overexpression of the transport protein reduces intracellular drug concentration and decreases cytotoxicity.

Ee et al. found success in diminishing MDR by downregulating BCRP expression using an siRNA specific to the gene. The group found that BCRP mRNA and protein levels significantly decrease twenty-four hours after siRNA transfection. They then performed cytotoxicity analyses on the siRNA-transfected cells to determine if there was an increase in sensitivity to chemotherapy drugs, specifically the antineoplastic agents mitoxantrone and topotecan. While mitoxantrone and topotecan have been found to be initially successful, their effectiveness decreases over time with MDR. The cancer cells were found to be sensitized to the two tested BCRP substrates. In addition, the cancer cells had enhanced intracellular accumulation of the chemotherapeutic drug. These results support siRNA deliveries as a potentially viable technique to combat MDR during chemotherapy.

Breast cancer is a significant and deadly disease in many modern societies. Breast cancer, like other cancers, is often initiated from an overexpressed gene leading to hypermorphic proteins and unregulated cell proliferation. Current chemotherapeutic substances can induce apoptosis and prevent cancer cells from proliferating and metastasizing. However, clinical practitioners using these strategies face difficulties with drug toxicity and cancer cells' ability to develop resistance to the drugs through increased expression of proteins that remove toxic compounds. RNAi appears to safely neutralize the proliferation, migration, and differentiation of cancer cells by selectively knocking down gene expression. RNAi can be exogenously transfected as dsRNA to be processed by Dicer into siRNA. The RISC-associated Argonaute will then bind to the siRNA, and the complex will facilitate endonuclease activity on a complementary mRNA strand to target specific gene expression for mRNA degradation or translational inhibition. Researchers have taken advantage of RNAi's knockdown capability to combat MDR and enhance the effectiveness of different chemotherapeutics.

The advancement of RNAi versatility could potentially be applied to combat all types of cancer. The ability to downregulate specific gene expression and enhance the performance of other drugs immensely increases the capacity of personalized drug design for cancer care. A system in which personalized drug design is commonplace would enable researchers to find the genotypic mutation specific to a tumor cell. Consequently, researchers could design an siRNA to target the exact mutation responsible for the cancer. It is likely that the world of oncology will revolve around genetic techniques, and RNAi has been the champion of the current era.

For more information about gene manipulation, check out the 2004 article "Unlocking the potential of the human genome with RNA interference" featured in volume 431 of *Nature*.