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RESEARCH HIGHLIGHT

Selective cell death mediated by small conditional RNAs: a novel therapeutic approach to cancer therapy

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Targeted molecular therapy has been an elusive goal in the treatment of neoplastic diseases. While chemotherapy has improved the outcome of many cancers, a non-selective cytotoxic approach invariably causes damage to normal cells, resulting in side effects and limiting treatment efficacy. This is evident in the evolution of treatment for castration-resistant prostate cancer (CRPC). For instance, while mitoxantrone, docetaxel and cabazitaxel have all shown therapeutic activity and have been FDA-approved for use in CRPC, response rates and duration of benefit remain limited and side effects associated with treatment are significant. Thus the development of specific targeted therapeutic agents which minimize toxicity to normal cells while killing malignant cells has been a high-priority endeavor. Sipuleucel-T, the first therapeutic cancer vaccine to demonstrate effectiveness in a phase III clinical trial, uses native antigen-presenting cells after incubation with a novel prostate cancer antigen (prostatic acid phosphatase), inducing an immune response against castration-resistant cells. Kantoff *et al.*[1] demonstrated that treatment of metastatic CRPC patients with sipuleucel-T resulted in a 22% reduction in the risk of death, and a 4.1-month improvement in median survival, compared with placebo. In addition, most treatment-related adverse events were transient and of low grade. Hence, increasing treatment target specificity may provide a desirable therapeutic effect while minimizing unwanted adverse events, in this case by inducing a cancer-specific immune response.

The discovery that gene expression can be controlled by interfering with messenger RNA (mRNA) has encouraged the development of targeted molecular therapy against various diseases.[2] Through base pairing with mRNA, technologically engineered double-stranded RNA can trigger silencing of complementary mRNA sequences. Such 'interference RNA' or RNAi has the ability to alter gene expression and has revolutionized research efforts in targeted molecular therapy. An example that has entered the clinic is bevasiranib, a vascular endothelial growth factor-targeted small inhibitory RNA (siRNA), currently in phase III trials of macular degeneration. Despite its therapeutic potential, however, a number of issues need to be addressed. For instance, genome-wide monitoring in microarray experiments has clearly demonstrated that siRNA-treated cells have off-target silencing of a large number of genes.[3] In addition, some synthetic siRNAs contain sequence motifs which can trigger unwanted inflammatory responses.[4]

Seeking to overcome some of these shortcomings, Venkataraman *et al.*[5] formulated a method of therapeutic regulation that is conditional, using small conditional RNAs (scRNAs) that activate protein kinase R-induced apoptosis only upon detection of a targeted cancer mutation.[6] The authors designed three transducers of a hybridization chain reaction, each consisting of two scRNAs, which recognize mRNA sequences in three specific human cancer cell lines: the endothelial growth factor receptor commonly found in glioblastoma, EWS/FLI1 in Ewing Sarcoma and the TPC/HPR fusion located on t(6;16)(q22;q12) in prostate carcinoma. Within the cancer cell, hybridization of the targeted cancer marker induces a chain reaction resulting in the activation of a protein kinase R-induced immune response and cell death. Selectivity for the cancer cells was high, with a 20- to 100-fold reduction in the population of malignant cells. In addition, the investigators confirmed the proposed mechanism of action *via* a series of experiments. For instance, incubation of the target transducer and a control cancer cell line that produced wild-type transcript did not result in cancer cell population reduction, confirming selectivity for mutant transcript-triggered cell death. The authors concluded that unlike conventional RNAi, scRNA performs mechanical transduction, functionally decouples diagnosis and treatment, and is conditional.

Despite possible advantages regarding this approach, the proposed scRNA faces several challenges as investigators attempt to bring this technology to clinical treatment of cancer. First, despite enhancing selectivity by decoupling diagnosis and treatment, scRNAs (like siRNAs) may cause off-target effects and induce pro-inflammatory responses *in vivo*.[6] Second, introducing ectopic scRNAs into cells may disrupt the intrinsic cellular machinery. Grimm *et al.* observed such an effect when mice receiving targeted siRNAs died from liver damage associated with reduced expression of liver specific micro-RNAs.[7] Third, given the variability of genetic abnormalities observed in solid tumors, targeting a single RNA sequence to induce apoptosis may not be ideal. For instance, expression of t(6;16)(q22;q12) in metastatic prostate adenocarcinoma is limited to a subset of cancers and thus may benefit only a small group of patients. Thus, tumor genotyping to program patient-specific scRNAs may be needed for this method to be effective. Fourth, effective *in vitro* delivery remains a significant obstacle.[8] Due to its negative charge, target scRNAs cannot cross into cells without further manipulation. Even while several approaches had been shown to be effective, therapeutic dosage delivery will have to be carefully tailored.

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This study highlights the potential of RNA therapeutics, leading to conditional activation of transducers and consequently cancer cell apoptosis. Using modern RNA technology, the authors successfully generated a potential therapeutic approach that targets only malignant cells while not affecting normal cells. While further studies in animal models are needed, the goal of targeted molecular therapy in the treatment of cancer may move a step forward with such RNA-based therapies.

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