Silencing of Mortalin with siRNA as adjuvant for cancer immunotherapy (Ramot)

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Technology

A novel mode of treatment by inhibition of mortalin or mortalin synthesis as an adjuvant to antibodybased immunotherapy.
Novel siRNA was designed as blocker of Mortalin expression. The anti-mortalin siRNA binds specifically to mortalin mRNA and leads to its destruction. Upon transfection into cells, this siRNA reduced the level of expression of mortalin by 60-90%. This was shown with several cancer cells. Mortalin specific siRNA was demonstrated to augment sensitivity of cancer cells to killing by antibodies and complement and to impair growth of cancer cells in vivo.

The Need

In recent years, new monoclonal antibodies have been designed to target immune cytotoxicity to the tumor cell. This era of targeted therapy has brought to the clinics a handful of monoclonal antibodies, including Rituxan, designed for CD20-positive non-Hodgkin B cell lymphoma, Herceptin for breast tumors and more. The clinical and commercial success of such anticancer antibodies has created great interest in antibody-based therapeutics for hematopoietic malignant neoplasms and solid tumors.
However, due to the increased resistance of cancer cells to complement-mediated lysis, the clinical impact of those antibodies has been restricted to activation of antibody-mediated cell-cytotoxicity (ADCC) responses and activation of programmed cell death (apoptosis). Addition of complementmediated cytotoxicity (CDC) responses to the activity of these antibodies is likely to amplify the therapeutic potential of tumor-targeted antibodies.
Cancer cells are equipped with evasion and resistance mechanisms that support their growth in immune competent patients. To resist complement-mediated lysis, cancer cells eliminate the complement membrane attack complex (MAC) from their surface by membrane vesiculation and/or endocytosis. These mechanisms are also obvious obstacles to immunotherapeutic treatments. We showed that mortalin (mtHsp70) is selectively released from complement-treated cells and that mortalin can bind directly to complement C9 and C8, the major MAC constituents. Mortalin is frequently up regulated in tumors, while over expression of mortalin in normal cells considerably extended their lifespan, and reduced mortalin levels in immortalized cells caused growth arrest. Our in vitro studies demonstrated that after inhibiting or silencing mortalin expression, tumor cells become increasingly sensitive to antibody-dependent complement-mediated lysis.

Potential Application

- A novel treatment for various cancers
- An adjuvant for antibody-based therapeutics for hematopoietic malignant neoplasms and solid tumours such as Rituxane, Herceptin, Mylotarg, Erbitux, Avastin and more.
- An adjuvant for antibody-based therapeutics for autoimmune and lymphoproliferative diseases (for removal of auto-reactive cytotoxic cells and lymphoproliferating cells).

Stage of Development

In vitro studies demonstrated that inhibition of mortalin with specific antibodies or by silencing of mortalin with siRNA augmented sensitivity of cancer cells to killing by antibody and complement. Treatment with specific siRNA reduced the level of mortalin in EL4 (T-lymphoma) cells to 30% of control as determined by Western blotting.

The effect of in vivo mortalin siRNA on tumor immunotherapy was tested in mouse models of cancer: Injection of the transfected cells subcutaneously or intravenously to C57Bl/6 mice resulted in reduced growth of EL4 cells subcutaneously and within the circulation as compared to non-specific

scrambled siRNA injected cells.
Additional in vivo studies demonstrated reduction of tumor size following injection of siRNA directed to mouse mortalin as compared to non-specific scrambled siRNA injection.
Combined treatment with anti-tumor antibody and mortalin specific siRNA led to complete regression of the tumor in 8/8 mice within 11 days. In the control group, treatment led to regression in 4/8 mice within 18 days, and 4/8 mice had a progressive disease till day 33 which would have most likely lead to their death.
Some of these data was confirmed with human cancer cells K562 and Raji grown subcutaneously in athymic nude mice.

Patents
Mortalin siRNA:
METHODS OF TREATING CANCER BY MODULATION OF MORTALIN
US patent: 8,293,716
DOWN-REGULATION OF MORTALIN BY siRNA
US patent: 8,470,793
Mortalin Peptides:
PCT patent applications pending

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