Novel Therapeutic Protocol in the Research of Breast Cancer Treatment - Targeting the Border

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Abstract

The complex signaling pathways and interactions inside and among cells which are responsible for the appearance and evolving of malignancy have a distinct and well separated border, the translation process, where the signals of a mutant and dysregulating genome are becoming specific proteins evolving to the malignant phenotype. We selected as translation target the Fragile X –Mental Retardation Protein (FMRP) of the breast cancer cell, a protein of whose role in translation has not clearly elucidated yet. Triple negative breast cancer cell lines (BCCL)-(BT20, MDA-MB-231, Hs578 T) were transfected by plasmid with mutant FMRP at progressively increased CGG triplets (from 45 to 200 repeats at a one by one CGG repeat step up process) and the production of the mutant protein was verified with the SUNSET method and Western blot analysis. Afterwards, the impact in protein synthesis inhibition and apoptosis induction of the transfected BCCL in relation to normal epithelial breast cell lines (NEBCL) was evaluated. Preliminary in vitro results showed slower rates of protein synthesis and higher apoptotic rate of the 78 CGG & 84 CGG repeat mutant FMRP transfected BCCL in compared to NEBCL, while for all other transfected BCCL no statistically significant differentiation was found.

Key words: *Breast cancer; therapeutic protocol; targeting the border*

Introduction

The complex signalling pathways and interactions within and among cells responsible for the appearance and development of malignancy have a distinct and well-separated border. The border where the “thoughts” - signals of a mutant and dysregulating genome of the cancer cell - take shape to become specific proteins evolving into the malignant phenotype (invasion, stromal dysregulation, immunomodulation and metastasis).

Targeting the beginning (membrane receptors, signalling pathways, second messenger RNAs etc.) or the end (growth factors, MMPs, angiogenic factors etc.) of this process has not yet led to definitive treatment (with few exceptions).

The new therapeutic target may be the border that the malignant genotype needs to overcome, in order to become a phenotype. This border is the translation process performed within the ribosome, the endoplasmic reticulum and the Golgi apparatus organelles. Targeting the malignant cell at various stages of this process, from the beginning of the formation of the ribosome, through mRNA interaction, until the end of the exit from the Golgi apparatus of the polypeptides responsible for the malignant phenotype may be a novel therapeutic approach against breast cancer research.

Initial design of methods: Since there are issues that need to be overcome, in order to efficiently target the border, the protocol is broken down further into seven branches, as described below:

1. Search for structural differences between the border organelles (BO) of a normal breast cell and those of its malignant variant, in order to find specific therapeutic targets, thus minimizing side effects. This can be done by thoroughly examining those organelles through electron microscopy with statistically significant repeatability of results.
2. Target the beginning of ribosome formation by inhibiting breast cancer cell-specific eIF4e factors, such as eIF4A3 [1,2], or other antigens from the BO of breast cancer cells found to have structural differences from the above branch. These specific antigens are then injected into mice and the antibody-forming cells are isolated from the spleen and fused with cultivated tumour cells to form hybridomas which are screened for antibody production. Antibody-producing hybridomas are cloned, and finally antibodies are isolated for cultivation and further study of their therapeutic effects in a randomized clinical trial.
3. Analyse with X-Ray crystallography which of the Fragile X Mental Retardation Protein (FMR1) isoforms is over-expressed within breast cancer cells [3]. Subsequently, determine whether there is competition between those isoforms and the mutant protein (and at which CGS sequencing) at the FBS-G quadruplex structure of the mRNA, which in consequence inhibits translation initiation [4]. Prospectively, establish whether using

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mutant FMR1 protein (and at which CGS sequencing) by selectively inserting it into the cancer cells (e.g. by using liposome vectors) could be implemented as a novel therapeutic approach.

4. Create transgenic mice groups [5] inserted with the mutant (Fragile X) FMR1 gene in various amounts of CGG repeats (5-44 repeats: control group; 45-54 repeats: group 1; 55-200 repeats: group2; 300-350: group 3; and 350-500: group 4). Afterwards, each group will be injected with the triple negative breast cancer tumour cells. Results regarding tumour progression and metastasis are histopathologically graded and statistically analysed for significance ($p < 0.05$) and safety (power $> 80\%$), thus providing evidence for further study.
5. Gene therapy of breast cancer [6] stem cells with retroviral transport agents able to transport genes, their product into the cells inhibits protein synthesis. Mutant FMR1 gene in alleles, were previously found that are tumour-suppressing but not related with full blown Fragile X- phenotype, within viral vectors with tropism for specific organs. For instance, HCV viral vectors carrying this gene injected directly into the hepatic artery may be used for liver metastasis treatment.
6. Co-culture of breast cancer cell lines [7] with agents e.g. micro-organisms formerly used to develop antimicrobial chemotherapeutics targeting the ribosome (e.g. aminoglycosides) and chemically alter the structure of the substance isolated in order to achieve inhibition of cancer cell ribosomes only.
7. Develop a fluorescent agent for breast cancer cell uptake only, thus allowing emitted radiation to be detected and used by a laser machine in a one-by-one cancer cell detection and destruction process for obtaining clear margins, in cases of local advanced disease or thoracic wall recurrence.

Note: This protocol was constituted in Athens by Dr. Panagiotis Bouras on 07/03/2016. The above-described methods may be reassessed, modulated, altered or substituted with newly developed techniques or others suggested by fellow scientists participating in the study, and always

according to the progress or specific discoveries during the course of this study. The central idea of this study, as described in the introduction and the initial design of methods section, shall irrevocably not alter. The final results of this research study that may be used by pharmaceutical companies or other institutions for further research or medication development for human treatment should always carry the original signature of Dr. Panagiotis Bouras M.D. Ph.D. as seen below:

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