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The Cancer Control Challenge:
Better Understanding It to Better Face It

PIWIL1-REGULATED CANCER TESTIS ANTIGENS MAGEC3 AND MAGEA6 AS POTENTIAL TARGETS FOR ANTINEOPLASTIC IMMUNOTHERAPY

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Introduction: Cancer presents a high mortality rate, related to the biology and aggressiveness of the disease itself, but also to poor response or adverse effects related to therapy. One strategy to mitigate this problem is immunotherapy, which recognizes specific targets in neoplastic cells and induces cell death through the immune system. In the search for tumor markers, there is the possibility of identifying targets that are more highly expressed in tumors and less abundant in healthy cells. In this regard, cancer/testis antigens (CTAs) represent a group of tumor-associated antigens that are normally expressed in immune-privileged tissues, such as the testicles, but present aberrant expression in various types of cancer, especially in the more advanced stages. Functionally, CTAs are implicated in the self-renewal and differentiation of stem cells in their respective lineages. Aberrant expression profiles of CTAs are associated with cancer development and can be used as biomarkers and targets for immunotherapy. Therefore, understanding the regulation of the expression of these antigens may contribute to the understanding of the carcinogenesis process and the prediction of targets for immunotherapeutics. In this context, it is known that the *PIWIL1* gene (piwi-like RNA-mediated gene silencing 1) encodes a key regulatory protein in transcription and translation, with expression almost exclusively in the testicles, overexpressed in cancerous tissues, stem cells, and germ cells, but absent in normal tissues. **Objectives:** Thus, this study sought to investigate the possible role of PIWIL1 in modulating the expression of CTAs. **Methods:** For this, *PIWIL1* knockout was performed by CRISPR-Cas9 in the AGP01 cell line (Metastatic Gastric Adenocarcinoma) established from cells present in ascitic fluid. The gene expression profile of AGP01 cell lines with and without *PIWIL1* knockout was obtained using a microarray assay, and the data were concatenated and described through software and then subjected to Levene's and Student's t-tests, considering a critical alpha of 0.05. **Results:** *PIWIL1* gene knockout was observed through the addition of seven adenines, resulting in a subsequent change of all amino acids immediately following the insertion point and a premature STOP codon. Among the differentially expressed CTAs, *MAGEC3* and *MAGEA6* show significant reduction in gene expression and relevant biological impact, possibly being directly or indirectly associated with the action cascade

of the *PIWIL1* gene. *MAGEA6* acts as a ubiquitin ligase activator that functions as an autophagy repressor and can also stimulate the ubiquitination of the p53/TP53 protein and play a role in tumor transformation or aspects of tumor progression. Meanwhile, *MAGEC3* exhibits antigenicity and is associated with stress-induced cell cycle arrest and reduced expression of DNA repair genes. **Conclusion:** Therefore, it is suggested that the two CTAs are potential candidates for antigenic immunotherapy, with a possible form of individualized treatment for patients with gastric cancer with *PIWIL1* overexpression.

Keywords: *PIWIL1;* Gastric cancer; Cancer-testis antigens