



Thymidine Kinase 1: Making Its Mark In Immunotherapy

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Abstract

Cancer is one of the leading causes of death worldwide. In the U.S. alone, almost 2 million people will be diagnosed with cancer each year and just over a quarter of those diagnosed will pass away from the disease. Skin cancers are the most common forms of cancer. Early detection of cancer and cancer biomarkers enables clearer understanding of cancer progression in a patient and more effective treatments in response to the disease. Clinically relevant biomarkers are not only tools for early diagnosis of cancer but may also prove useful as immune targets for various immunotherapies, such as monoclonal antibody-based therapy and chimeric antigen receptor (CAR) T-cell therapy. This review provides a brief overview of the rescue pathway enzyme thymidine kinase 1 (TK1) and its history and biology, as well as discusses its role as a biomarker and potential immune target.

Introduction

Cancer biomarkers are crucial for diagnosis and prognosis. One protein widely recognized as a clinically valuable biomarker and potential immune target is salvage pathway enzyme thymidine kinase 1 (TK1). With the discovery of DNA structure and replication methods in the 1950s, research since then has produced significant discoveries regarding TK1, its characterization and functions. The regeneration of thymidine for DNA synthesis and damage is accomplished in part by TK1¹. In 1983, Bradshaw et al. was able to sequence the TK1 gene and identify a section within the 5' end region that induced S phase activity². TK1 upregulation in the S phase of the cell cycle allows the enzyme to become an indicator for active cell proliferation³⁻⁵. Because of its upregulation in various cancers, TK1 is a significant biomarker for cancer and cancer stage⁴. While TK1 is known to be involved in cancer progression, there is limited research specifically focused on the correlations between TK1 and the progression of skin cancers. One study does emphasize a correlation between serum TK1 and metastatic sites in melanoma, indicating that the enzyme may be involved in the progression of melanoma metastasis⁶. This mini review expands on a general history of TK1 and concludes with an exploration of recent discoveries and further applications for TK1 with regard to novel immunotherapies.

Role of TK1 as a Salvage Pathway Enzyme

TK1 is a DNA salvage pathway enzyme that is responsible for recycling and regenerating thymidine for DNA synthesis. Generally, the de novo pathway is also utilized by cells for regenerating nucleotides; however, the salvage pathway is less energy expensive. Thymidine crosses the cell membrane and enters the cytosol via facilitated diffusion and is then phosphorylated into its

monophosphate form (dTMP) by TK1⁷. Following this first step, other enzymes are recruited to convert dTMP to deoxythymidine triphosphate (dTTP), its triphosphate form. Nucleotides such as dTTP enter the nucleus via passive transport⁸. In addition to DNA synthesis, TK1 plays an important role in managing DNA repair because it generates a supply of dTTP that is readily available to replace damaged nucleotides. DNA damage caused by radiation or chemotherapy often results in increased TK1 levels¹. TK1 is essential to repair DNA damage, and required to form nucleotides destined for cellular repair outside of S phase^{1,9}. In fact, TK1 depletion in cells with DNA damage often results in cell death⁴.

Regulation of TK1

TK1 plays a critical role in cell cycle regulation. Protein dysregulation and mutations may promote the breaking of cell cycle control and induce tumorigenesis¹⁰. Adler et al., performed some early studies indicating that TK1 cytosolic levels peak during S phase¹¹. During periods of DNA damage and normal cell function, mRNA levels of TK1 are controlled by the RB/E2F pathway, which is known to regulate transcription at the promoter site¹². Bradshaw et al. discovered a 16-kilobase region, located on the 5' end of the TK1 gene, responsible for TK1 activity during S phase². Additionally, there are important regulatory elements located in the TK1 gene's coding region and a 70 bp region within the promoter that controls TK1 expression¹³. Also, TK1 normally exists as a quiescent dimer and as a tetramer when active during S phase¹⁴. TK1 tetramer activity is allosterically controlled by dTTP levels. When dTTP binding occurs, the binding site for ATP and thymidine on TK1 is blocked, which prevents thymidine phosphorylation¹⁵. However, the phosphorylation of serine residues during post-translational modification induces TK1 activation¹⁴. Overall, TK1 has proven to be an important cell cycle protein that will continue to be researched to better elucidate its many roles.

TK1 As a Biomarker for Cancer

The discovery of TK1 was introduced in the 1960s¹⁶. Since then, elevated levels of TK1 have been shown in various studies that focused on the sera of many different cancer types, including lung, colon, breast, and prostate¹. In one study done by Alegre et al., they showed that TK1 levels were elevated in lung cancer. Their results reflected that TK1 was significantly elevated in all lung cancer samples. Patients with stage I (n=16) and stage II (n=17) cancer showed higher TK1 levels compared to their controls¹⁷. A study done on serum TK1 (sTK1) levels during neoadjuvant chemotherapy (NACT) for early breast cancer (BC) longitudinally assessed TK1 as a reputed long-term biomarker in BC, both at baseline and following short-term exposure to NACT. Their results showed that

TK1 levels increased compared to baseline levels 48 hours after administration of the chemotherapy¹⁸. A clinical test done by Larsson et al. evaluated sTK1 in metastatic breast cancer (MBC) patients as a tool for disease prediction and treatment monitoring. They monitored 142 women with MBC and found that higher sTK1 levels correlated with worse progression-free (PFS) and overall survival (OS) at baseline levels. During 1st line systemic therapy (ET, ChT or HER2 targeted therapy), they noticed that changes within 3 to 6 months significantly correlated with PFS and OS¹⁹. Along with this finding, a recent discovery by Liu et al. showed that the knock down of TK1 in cells reduces proliferation, invasion, and cell migration. They studied these effects on thyroid carcinoma tissues and developed strong evidence between TK1 levels and cancer prognosis²⁰.

In a study done by Weagel et al., TK1 is confirmed to reside as monomeric and dimer forms that function on the cell membrane. They confirm that TK1 is upregulated and contained on the plasma membrane of Burkitt's lymphoma, acute promyelocytic leukemia, T cell leukemia, and acute lymphoblastic leukemia. This starkly contrasts their findings on normal resting or proliferating lymphocytes where TK1 upregulation does not occur²¹. As elevated expression of TK1 has been shown to be present in multiple cancer types, cancer development, and immune-suppression, TK1 can be developed as a beneficial tool as a tumor biomarker when testing for cancer and disease management. Its potential as a biomarker also leads to possibilities for the targeting of TK1 with immunotherapeutic treatments.

Additional Factors in Immune-Targeting and Immunotherapy Administration

In conjunction with the study of cancer biomarkers and other biological mechanisms to prevent, diagnose, and treat cancer, it is important to understand pathogenic interactions among environment, host, and tumor. These interactions lend to the understanding of the molecular pathology and epidemiology of cancer and aid in determining how a patient will respond to immunotherapy.

A major barrier for immunotherapies is the tumor microenvironment (TME). The TME is made up of cancer cells, extracellular matrix components, secreted chemokines and cytokines, signaling molecules, and infiltrating immune cells, and is involved in immune cell recruitment and activation, drug resistance, and tumor progression^{22,23}. The TME influences immunosuppression through the secretion of chemokines and cytokines by tumor-associated macrophages, regulatory T cells (Tregs), fibroblasts, and other tumor cells^{24,25}. Rapidly proliferating tumor cells increase their nutrient uptake, produce inhibitory metabolites, and convert to an acidic environment through the production of lactic acid and carbonic acid during aerobic glycolysis and oxidative

phosphorylation^{25,26}. The metabolically hostile TME forces immune cells to compete for nutrients and makes it difficult for cells to survive in their hypoxic and acidic surroundings^{24,26,27}. This interactive biological ecosystem is also affected by aging, diet, drug and alcohol consumption, adiposity, diabetes, physical exercise, and other genetic and epidemiological factors²⁸. Navigation of the TME is crucial for adequate administration of immunotherapies.

In conjunction with the TME, continuous interaction with the immune system causes the human microbiome to also have a role in immunotherapy efficacy. Monitoring and recognition of microbes is largely performed by the innate immune system, thus, microbes and immune cell populations can be found in close proximity²⁹. Evidence indicates that variation in the human microbiome can have carcinogenic effects^{28,30}. These microflora alterations may include biochemical changes. One such change is the hypothesized metabolism of Thymidine Kinase by gut microflora. Data from one study shows that Thymidine Kinase (TK) activity is reduced by 50% in normal mice compared to germ free³¹. TK activity is known to be involved in cancer development, thus, through interaction with TK and other possible enzymes involved in cancer progression, microflora is suggested to be involved in tumor progression³⁰. Evidence suggests that tumor phenotype may also be partially determined by specific microorganisms present^{28,32}. Similar to the TME, human microbiota are influenced by diet, alcohol use, medications, and other lifestyle factors³³.

The TME and human microbiome represent various factors involved in carcinogenesis and characterize the influence that interactions between environment, host, and tumor have on cancer progression. Continued research of these interactions is essential for effective recognition of cancer biomarkers and biomarker targeting in immunotherapies.

Recent Discoveries and Further Applications for TK1

In the last decade, immunological advances have allowed tumor biomarkers like TK1 to garner marked attention as a diagnostic, prognostic, and therapeutic targets in the clinical setting. Specifically, TK1 expression has been demonstrated to increase in lung, breast, and colon tumor cell lines¹ indicating that patients presenting any of the aforementioned cancers as potential candidates for TK1-targeted immunotherapy. In an extensive review, Bitter et al.¹ references a variety of studies that identify TK1 as a potential biomarker found in lung adenocarcinoma³⁴, thyroid carcinoma²⁰, as well as associated with a variety of other tumor-progression mechanisms³⁵. An additional study done by Miran et al. identifies TK1 as a key enzyme in nucleotide synthesis and proliferation of malignant

melanoma. Utilizing TK1 as a target both *in vitro* and *in vivo*, this preclinical study suggests TK1 as a potential target for “highly efficient and selective” radioactive clinical treatment and possibilities for TK1 targeting in melanoma patients³⁶.

Monoclonal antibodies primed to TK1 are utilized as both diagnostic and therapeutic agents for various cancers, including malignant skin cell carcinoma. While approved chemotherapeutic agents have slow response rates with metastatic melanoma, monoclonal antibodies against traditional antigens for the disease induce a notably faster durable response rate³⁷. This can be attributed to high target specificity and the accompanying immunotherapeutic effect depending on the antigen of choice. Velazquez et al. developed and evaluated a set of monoclonal antibodies against TK1. Binding of the antibodies was confirmed by Western blot in recombinant protein, cancer serum, and cell lysate, suggesting good affinity³⁸. These results combined with the potential efficacy associated with monoclonal antibody treatment in melanoma patients nominate TK1 as an additional target enzyme utilized in treatment.

Chimeric antigen receptor (CAR) technology represents a novel immunotherapeutic class consisting of cloned leukocytes obtained from a diseased host for which the therapy is intended. CAR T-cell therapy is most effective when utilized in presentation of B-cell Leukemia and Lymphoma, as these require systemic attention for remission to occur³⁹. Similarly, the diffuse nature of disease in patients presenting with metastatic melanoma suggests clinical potency in utilizing CAR T-cells with anti-TK1 receptors. A limitation associated with CAR T-therapy is nullification by the microenvironment typically associated with tumor. Particularly new development and discussion of CAR-Macrophage therapy, however, shows promise. Macrophages are known to aggregate around and interact with the tumor microenvironment⁴⁰, facilitating a more focused immunotherapeutic reaction against the desired solid tumor while mitigating patient harm, indicating compatibility as a vehicle in TK1-associated immunotherapy.

Conclusions

As technological advancements are made, there is increased opportunity for further discovery and application of clinically relevant biomarkers in the diagnosing of cancers and progression of effective treatment. When upregulated, the salvage pathway enzyme Thymidine Kinase 1 is shown to be in direct correlation with cancer stage. In this manner, TK1 provides a platform as a biomarker for the tracking of cancer progression and possible immunotargeting during treatment. Substantial research already implementing TK1 as a biomarker and immune target for various cancers has

produced positive results. Further research of biomarkers such as TK1 has potential to improve the diagnosis and prognosis of cancers.

Conflict of Interest

There are no conflicts of interest to report.

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