Thymidine kinase: diagnostic and prognostic potential
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Thymidine kinase is a cell cycle-dependent marker that can be detected in the serum of patients diagnosed with many different types of cancer. Serum levels of thymidine kinase have also been shown to reflect the progression of cancer as well as an indication of the efficacy of chemotherapeutic intervention. A new monoclonal antibody assay for thymidine kinase has been developed which is capable of detecting thymidine kinase in both serum and tumor tissue. Thymidine kinase assay kits should be available at low cost and could serve as an effective low cost test for the detection and progression of many types of human cancer.


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KEYWORDS: cancer marker, detection, diagnostic, monoclonal antibody, TK, thymidine kinase, prognosis

Today the lives of millions of individuals have been affected both directly and indirectly by cancer. According to data from the American Cancer Society for the year 2001, more than 550,000 Americans are expected to die from various forms of cancer. Cancer is second only to heart disease as the leading cause of deaths in the USA. The same report states that 1.2 million new cases of cancer are expected to be diagnosed in 2001 in the USA — this data did not include carcinoma in situ or basal and squamous cell skin cancers, which would double the current estimate.

Until a few years ago the word ‘cancer’ was often regarded as synonymous with death. Cancer is still widely misunderstood, the general public is slowly coming to understand that cancer is some 400 different diseases that can be controlled and may even be curable in instances when diagnosis is early and followed appropriate clinical treatment. Early diagnosis of cancer can often mean the difference between survival and death. Survival rates dramatically decrease if the cancer becomes metastatic and spreads into the lymphatic/vascular system and disseminates throughout the body. This can lead to invasion of other tissues and eventually leads to organ failure and subsequent death of the patient. When cancer reaches these advanced stages the prognosis for successful treatment is often very bleak. Early detection offers the best opportunity for treatments that have the highest survival rate, i.e., localized excision, followed by radiation or a chemotherapeutic regimen. For example, the 5-year survival rate for cervical cancer is 92% if diagnosis is obtained when tumors are still localized and the 5-year survival rate for prostate cancer is 100% when diagnosed at the localized stage. When cancer is not detected until late in the course of the disease, the options and success of treatment dramatically decrease. Some cancers are so aggressive that if left undetected and untreated the estimated life expectancy for the patient can be measured in months. The dependence of early detection of cancer for patient survival points to the critical need for new and accurate detection methods. Numerous methods for detecting cancer are currently employed using assays that detect the presence of specific tumor markers. Well-known tumor markers include carcinoembryonic antigen (CEA), BRCA-1 and -2, prostate specific antigen (PSA), Epstein-Barr nuclear antigen (EBNA) and the pap smear for human papillomavirus 16 (HPV-16) cyto logical alterations.

In addition to assaying for known tumor markers, targeting continually dividing cells for possible cancer markers has also been
pursued since there are very few cell types in the body that divide at the same frequency as cancerous cells. This approach could lead to the development of an accurate, non-invasive method of detecting the presence and potential of a malignancy. In fact, it was this approach that led to the discovery of a new tumor marker, thymidine kinase (TK), that has been shown to have widespread use as a diagnostic marker for cancer detection and prognosis.

**TK: a new cancer diagnostic marker**

**History of TK**

In the 1950s it was determined that $^{15}$N-labeled thymidine could be incorporated into DNA [4]. It was later observed that it was necessary for thymidine to be phosphorylated before it could be incorporated into the DNA [6]. It was further observed that thymidine could be incorporated into the nascent polynucleotide only after being converted to a triphosphate form (dTTP) [5]. The synthesis of this phosphorylated nucleoside requires multiple steps from distinct kinase enzymes. The first was shown to be TK (thymidine-5-phosphotransferase EC 2.7.1.21), which catalyzes the conversion of thymidine to thymidine monophosphate (dTMP) in an ATP-dependent reaction.

The biosynthesis of dTMP via TK is not essential for DNA synthesis. The de novo synthesis of dTMP is achieved through a complex series of reactions, in which separate and communicates phosphoryl donor. In contrast to the decreased activity of TK1, TK2 is only 10–15% of total activity – when CTP is the phosphate source. However, TK1 activity is dramatically decreased – retaining only 10–15% of total activity – when CTP is the phosphate donor. In contrast to the decreased activity of TK1, TK2 is rapidly inhibited, retaining 86–93% activity, with CTP [5].

**Characteristics of TK**

Molecular biology has grown in its ability to characterize the intricacies of a disease such as cancer. Elucidating the specifics of the TK1 gene, protein and promoter have benefited from this molecular revolution. TK1 has been isolated in many different cell lines and tissue types. Depending on the isolation method, there have been reports of varying sites of the protein. These sites range dramatically from small monomers to 500 kDa protein complexes, all of which have been identified as having TK activity. The smallest functional gene for TK1 has been shown to be 1421 nucleotide base pairs; this yields a protein with a molecular weight of 25 kDa. It has been suggested that the catalytically active complex is larger. A 96 kDa protein from HeLa cells has been characterized by the use of purified cell extract. A large range of protein complexes exhibiting TK activity have been identified in the serum of patients suffering from various forms cancer. These complexes can vary greatly in size making identification of the basic functional subunit difficult. For example, a huge 730 kDa complex retained 80% of the TK activity when separated by gel filtration. A minor fraction of 58 kDa was also shared [7]. Under specific conditions (400 mM deoxythymidine; DTE) the large complex was broken down to the smallest molecular weight peptide. The same study also pointed out a significant difference in the stability of s-TK as compared to TK1 isolated from cellular extract [21].

Another important characteristic of the TK1 protein is the increasing understanding of its regulation during the cell cycle. It is well known that TK1 activity increases at the G1/S boundary rapidly declines when approaching M phase. Regulation of mammalian TK1 gene expression has been reviewed [12]. It has also been shown that the TK1 is under tight regulation at several points, namely transcriptional and post-transcriptional levels [13]. There are also sequences in the protein that seem to regulate its activity during the cell cycle [14]. It has been suggested that the TK1 promoter has binding sites for factors that are associated with the cell cycle. TK1 mRNA levels have been affected by several factors including E2F, polyomavirus large T antigen and possibly c-myc, h-ras and p21 (currently under investigation in our laboratory). It has long been observed that increases of TK in the serum of humans (s-TK) have been associated with various forms of cancer. This review will summarize the use of s-TK as a powerful marker for the presence of malignancy and its diagnostic potential in several types of cancers.
\( \text{TK in hematological cancers} \)

Increases in s-TK levels have been associated with multiple hematological neoplastic diseases specifically acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), Hodgkin's disease (HD), non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL), multiple myeloma (MM) and chronic myelogenous leukemia (CML). The diagnostic and prognostic potential of s-TK for some of the above-mentioned malignancies are further examined below.

\( \text{Non-Hodgkin's lymphoma} \)

Several studies have addressed the potential of s-TK as a tumor marker with diagnostic and prognostic capabilities in NHL [14–16]. Pretreatment s-TK levels were found to be a powerful discriminator of disease stage. s-TK levels of pretreatment patients were also significant in relation to determining the grade of the malignancy (low, med, high). s-TK levels also give insight to prognostic information, not only concerning response to treatment but with regard to predicting patient survival. s-TK levels were especially useful in predicting the disease course in low-grade NHL; greater than doubling the survival rate is around 90% [24]. In a study evaluating the prognostic values of several biochemical markers: s-TK, lactate dehydrogenase and CEA, s-TK was shown to be the only marker potentially useful for predicting extent of disease spread and prognosis of individual cases. s-TK levels were again capable of distinguishing stages of SCCL but were not able to separate malignancy subgroups as was previously shown possible with patients with NHL [26]. Another study suggested that the prognostic capability of s-TK is greater than that of neuron specific enolase and tissue polypeptide antigen in predicting tumor load and recurrence in SCCL [27].

\( \text{TK in small cell carcinoma of the lung} \)

Small cell carcinoma of the lung (SCLC) is an extremely aggressive and metastatic prone malignancy that if left untreated has an extremely short survival time. If diagnosed and treated early the survival rate is around 90% [26]. In a study evaluating the prognostic values of several biochemical markers: s-TK, acid phosphatase, PSA, neopterin, TK, osteocalcin, c-reactive protein and tissue polypeptide antigen) evaluated the acid phosphatase, PSA, neopterin, TK, osteocalcin, c-reactive protein and tissue polypeptide antigen) evaluated the ability of these markers to predict death of the patients from prostatic cancer. Using Cox's regression and multivariate analysis it was shown that s-TK was the best marker as a prognostic indicator. Some of the other markers reported values with high levels of false-positives or -negatives. PSA is currently the predominant tumor marker for prostate cancer. One of the major advantages of PSA is its ability to report the presence of residual disease after removal of the malignancy [26]. One of the drawbacks of this marker is its inability to differentiate between a malignant or benign lesion. Another problem with PSA is that some poorly differentiated tumors do not produce PSA, yielding potential false-negative results. Caution must be taken as the diagnostic abilities of PSA and s-TK could guide clinicians to a course of treatment, being able to identify the high-risk progressive disease patients from lower risk, redirecting the treatment (e.g., radiotherapy versus radical prostatectomy). There are however, s-TK levels can be clinically useful in distinguishing between MM and monoclonal gamopathy of undetermined significance (MGUS). Patients with MGUS have been shown to have significantly lower s-TK levels than those with MM [24]; although it was established that s-TK levels cannot be directly correlated with MGUS disease progression, it is useful in differentiating between MGUS and full blown MM [22]. s-TK levels in MM patients increased during the course of the disease and high pretreatment s-TK levels correlated with shortened survival of the patient [26]. Another benefit of monitoring s-TK levels in contrast to other cell proliferation assays – some of which require a bone marrow sample – is that s-TK levels can be determined via a noninvasive, easily acquired blood sample. TK has exhibited potential to be a powerful diagnostic and prognostic tool in hematological malignancies. Monitoring s-TK levels (particularly TK1 levels) in patients with these types of cancers can greatly aid the clinician in guiding a patient to the proper treatment for their disease.

\( \text{TK as a cancer marker} \)

A study comparing seven serum tumor markers (prostatic acid phosphatase, PSA, neopterin, TK, osteocalcin, c-reactive protein and tissue polypeptide antigen) evaluated the ability of these markers to predict death of the patients from prostate cancer. Using Cox's regression and multivariate analysis it was shown that s-TK was the best marker as a prognostic indicator. Some of the other markers reported values with high levels of false-positives or -negatives. PSA is currently the predominant tumor marker for prostate cancer. One of the major advantages of PSA is its ability to report the presence of residual disease after removal of the malignancy [26]. One of the drawbacks of this marker is its inability to differentiate between a malignant or benign lesion. Another problem with PSA is that some poorly differentiated tumors do not produce PSA, yielding potential false-negative results. Caution must be taken as the diagnostic abilities of PSA and s-TK could guide clinicians to a course of treatment, being able to identify the high-risk progressive disease patients from lower risk, redirecting the treatment (e.g., radiotherapy versus radical prostatectomy). There are however,
limits to using s-TK and PSA as prognostic tools in prostate cancer, s-TK alone could not distinguish between localized disease and disease that had spread to regional lymph nodes, but the use of these two markers together could distinguish true localized disease from metastasis in over 90% of the patients studied [31].

**s-TK in breast cancer**

Breast cancer has been one of the most studied malignancies in determining the prognostic potential of TK. s-TK has been shown to be of prognostic value in several different capacities. In a recent study of approximately 1700 primary breast cancer patients, monitoring TK levels taken from tumor samples was shown to correlate with disease-specific survival, local recurrence-free interval and distant relapse-free interval. The same study further explores the effectiveness of specific chemotherapies on patients and tumor TK levels. s-TK levels were also shown to reflect responses to therapy and prediction of possible tumor recurrence [36].

Recently there has been interest in enzymes that have similar substrates or products as TK namely: thymidylate synthase (TS) (de novo pathway) and thymidine phosphorlyase (salvage pathway). One recent study of 154 node-positive primary breast cancer patients showed a strong correlation of levels of TK and TS with survival of the patient. Although it was found that TS alone had no direct correlation with disease-free survival or overall survival of the patient, TS has been demonstrated to be useful in identifying the response of patients to chemotherapeutics, especially 5-FU [36]. This relationship was the focus of another study of 257 primary breast tumors from patients who received tamoxifen as their first chemotherapeutic treatment after diagnosis. These data showed a significant correlation with high tumor TK activity and the effect of tamoxifen therapy. They also concluded that high TS activity in the tumors correlated with an increased risk of recurrence for patients treated with tamoxifen. It has been shown that s-TK levels can distinguish between operable breast cancer and inoperable systemic cancer. Patients with operable breast cancer were monitored during treatment and a rise in s-TK levels correlated with development of systemic malignancy. s-TK prognostic limitations were manifest in as much as no detectable difference between patients with stage I (low), stage II (moderate), or stage III (high) tumors could be distinguished. Another limitation of s-TK was its inability to distinguish a correlation between ER status of the tumor. This ability would be valuable to the clinician in prescribing hormone replacement therapy to postmenopausal women who have had breast cancer. One study of 290 operable breast cancer patients showed that s-TK levels correlate with progesterone receptor status in pre/peri-menopausal patients and that s-TK was the only factor capable of predicting survival in post-menopausal women [33]. s-TK levels were compared to other prognostic markers for metastasis including: histological grade, nuclear DNA content, estrogen receptor and nodal status. It was shown that high s-TK levels were found to correlate with early stages of metastasis [36].

It has been observed that the increase in s-TK in breast cancer was largely due to an increase in the TK1 isozyme [34]. The mechanism for which TK1 or TK2 enters the serum continues to elude scientists. The two most accepted theories are: TK is released from cycling cells and accumulates in the serum, or it is released when tumor cells are destroyed, possibly by the immune system.

As mentioned previously the standard method of monitoring s-TK levels has been variations of a radio immunoassay. A recent study group report by researchers from the European Organization for Research and Treatment of Cancer reiterated the importance of proper MgCl2 and ATP levels used in the extraction of TK and dilution buffers used in the TK assay. This report reinforced the importance of proper concentrations of buffer components used to stabilize the enzyme and obtain reproducible, consistent results not only in serum levels but also in monitoring TK levels in tumors [35]. The TK assay has been used to identify TK levels in tumors of several types of cancers [36–38]. Although some problems associated with monitoring TK levels using a radio immunoassay have been overcome, it is still difficult to maintain consistent results and requires special trained technicians. These and other problems have pointed to the need for an immuno-based assay. Several labs have expressed the desire and intention of pursuing this endeavor but up until the mid1990s no one had created one. Our lab produced a monoclonal antibody to TK1 that has proved its usefulness and accuracy in an immunoassay. This antibody gives researchers the ability to distinguish an increase in serum levels of TK1 rather than simply an increase in total TK activity, which up until now was the only method possible. This assay has shown to correlate with the activity in s-TK1 and is able detect small changes of enzyme in the serum of cancer patients [39].

Having an immunoassay for TK1 will aid clinicians in monitoring the existence, progression and recurrence of breast cancer as well as many other malignancies.

**TK in other malignancies**

s-TK levels have been shown to correlate with prognostic indicators for many other different types of cancers. Squamous cell head and neck cancer (oral cavity, oropharynx and hypopharynx) cancers were main type of cancer that has been researched, but up until now few studies have been able to correlate the ability of s-TK levels to correspond to overall survival of the patient [36]. Researchers in Japan used RT-PCR to examine TK mRNA levels in tumors in order to investigate a correlation between TK and TS levels in patients with cervical carcinoma. TS is involved in de novo synthesis of pyrimidines and has been investigated as a prognostic indicator for this type of cancer [36, 40]. Colorectal neoplasia has also been investigated concerning its potential correlation with s-TK levels. It was shown that s-TK levels were elevated in advanced stages of carcinoma of the colon. Prognostic stages of this malignancy did not seem to correlate with serum levels of TK [40]. These data support the potential power of TK as a tumor marker for identifying early...
neoplasia and will aid in determining an appropriate course of treatment. In determining appropriate treatment, it should be recognized that higher levels of TK may hinder the effects of some chemotherapeutics and radiation [44]. An upregulation of TK was shown in cisplatin-resistant cells when compared to nonresistant parental cells [41]. Corresponding effects were seen in glioma cells that were TK-negative, those cells were found to be more sensitive to radiation [40].

It should be mentioned that other (noncancer) factors will also result in an elevated level of TK in the serum. The main causes for this transient elevation are: pernicious anemia and acute stages of herpes simplex virus induced disease. Both conditions are easily identified and therefore do not give false results when analyzing samples for s-TK levels.

Summary & conclusions

Even though all the regulatory mechanisms of TK are not yet fully understood, this enzyme has proved to be a powerful prognostic tool for many types of malignancies. Currently the TK1 monoclonal antibody is being used in immunoperoxidase and immunofluorescence staining of tissue samples. This will greatly aid clinicians in proper diagnosis and a more accurate prognosis for many malignancies. Along with the immunohistochemistry implications of this antibody, the technology to screen minute changes in protein levels in serum and tissue samples is now possible. From its discovery in the 1950s TK has proven itself to be a very powerful tool in the fight against cancer.

Expert opinion

The prospect of the availability of a noninvasive monoclonal TK1 assay as an indicator of both early cancer onset and clinical prognosis during treatment would be a significant advancement in the war against cancer. The widespread appearance of TK as an early cancer marker and the data suggesting its usefulness as a prognostic tool for the clinician could be a significant advancement in obtaining higher cancer survival rates. It is anticipated that the cost of TK kits would allow widespread clinical use.

Five-year view

The addition of a new cancer diagnostic test at low cost and wide use in cancer management could easily be envisioned within 5 years.

Key issues

• Serum TK1 level is a useful cancer marker.
• Elevated TK1 levels occur in a large number of different human cancers.
• Monoclonal TK1 antibody is a sensitive assay for TK1 levels.
• TK1 is a histological cancer marker.
• Serum TK1 levels can be used to monitor cancer progress.
• Assays for TK1 are noninvasive.
• TK1 assay kits would offer a low cost test for cancer markers.

References

Papers of special note have been highlighted:

O'Neill, Buckwalter & Murray


Website