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Short Commentary

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Standard WT1 Expression Using Immunohistochemistry

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Abstract

Although it remains difficult to treat advanced cancers affecting organs with distant metastasis, therapeutic cancer vaccination is conducted for the induction of an efficient immune response against tumor-associated antigens. Wilms' tumor 1 (WT1) shown to be the most potent tumor-associated antigens may have potentially strong therapeutic activity against cancers. WT1 expression using immunohistochemistry (IHC) can be evaluated on paraffin-embedded tissues and is proportional to mRNA levels. The standardized IHC would be useful for personalized cancer immunotherapy.

Keywords: Wilms'Tumor1; Cancer vaccination; Immunohistochemistry

Cancer vaccination is conducted for the induction of an efficient immune response against tumor-associated antigens in the acquired immune system. The vaccination therapies are principally attributed to the presence of tumor-associated antigens. Wilms' tumor 1 (WT1) was identified to be an attractive target antigen, widely detected in cancer, sarcoma, and leukemia. WT1 fulfilled immunological and clinical effectiveness with respect to therapeutic functions, immunogenicity, specificity, and oncogenicity [1].

We established the standard of WT1 expression using immunohistochemistry (IHC) of cell lines. First, mRNA expression of cell lines included WT1-positive cell lines, Panc-1 and MIA PaCa-2 (Riken Cell Bank, Tsukuba, Japan), and WT1-negative cell lines, HCT116 and MCF7 (Riken Cell Bank, Tsukuba, Japan), and was confirmed with real-time polymerase chain reaction [2]. Quantitative copy numbers of WT1 in Pank-1 and MIA PaCa-2 indicated $1.9 \times 10^{5/2}$ μ g RNA and 8.7 × 10³/ μ g RNA, respectively (Figure 1A). WT1 IHC of those cell lines revealed expression using a mouse monoclonal antibody (6F-H2, DakoCytomation, CA) [3] that was proportionate to the mRNA level (Figure 1A). In reference to those cell lines, WT1 expression was evaluated with immunohistochemical staining on paraffin-embedded tumor tissues. If WT1 expression was observed by IHC, tumor tissue was considered positive; if no expression was observed, it was considered negative. Human leukocyte antigen (HLA)-ABC antigen (class I, W6/32, DakoCytomation), HLA-DR antigen, alpha-chain (class II, TAL.1B5, DakoCytomation), and epithelial membrane antigen (EMA, MUC1, Clone E29, DakoCytomation) were also evaluated on the same tissues in parallel with WT1 staining.

IHC was adopted because clinical materials derived from formalinfixed paraffin embedded tissues were not adequate for quantitative PCR analysis. The specificity of WT1 expression by IHC was determined using positive cell lines (Panc1, MIA PaCa-2) and negative cell lines (HCT116, MCF7) which were confirmed by quantitative PCR analysis. The epitope of anti-WT1 monoclonal antibody (6F-H2) covers N-terminal amino acids 1-181, which includes the domain of HLA A*02:01- or A*02:06-restricted peptides (126–134: RMFPNAPYL). WT1-IHC of those cell lines showed proportional expression to their mRNA level. Therefore, WT1 expression applied to tissue specimens was semi quantitatively evaluated in comparison with the staining profiles of these positive and negative cell lines.

WT1 expression was detected in primary cancer cells, originally isolated from an autopsy case of pancreatic adenocarcinoma, shown in Figure 1B. HLA-class I and EMA were positive, while there was a lack



Figure 1: Wilms' Tumor 1 (WT1) expression. (A) Real time PCR and WT1monoclonal antibody. (B) Immunohistochemistry (IHC) of primary pancreatic cancer cells. *, Arrow head indicates cancer cells. (C) IHC of liver metastatic pancreatic cancer cells. *, Arrow head indicates cancer cells in hepatocytes.

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of HLA-class II on the same cancer cells (Figure 1B). In contrast, WT1 and HLA-class I indicated low expression in metastatic liver cancer cells from the same autopsy case, despite HLA-DR and EMA showing the same profile. This suggested heterogeneity of cancer cells from primary cancer cells to metastatic lesions (Figure 1C).

Antigenicity of cancer cells other than activation of immune suppressors [4-8] can be exploited to immune escape of cancer cells. As liver metastasis is known to be a poor prognostic factor for patients with pancreatic cancer [9], such emergence of WT1 and HLA-class I antigens would provide an advantage for cancer cells resistant to WT1specific cytotoxic T cells induced by cancer immunotherapy. It has also been reported that WT1 antigen in pancreatic cancer cell lines could be up-regulated by chemotherapeutic drugs, such as gemcitabine (2'2'- difluorodeoxycytidine, GEM) [10]. Consequently, the effect of GEM on cancer cells could ablate the immune escape mechanisms. The evaluation using IHC would be useful for personalized cancer immunotherapy targeting WT1.

Disclosure of Interest

All authors declare no conflict of interest that could be perceived as prejudicing the impartiality of the reported research.

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