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Review

WT1 AND MULTIPLE MYELOMA: A POTENTIAL IMMUNOTHERAPEUTIC APPROACH FOR HAEMATOLOGICAL MALIGNANCIES

Ilaria Dulcamare [1], Grazia Scandura [1], Enrico La Spina [2], Daniela Cambria [1], Tatiana Zuppelli [1], Vincenzo Bramanti [3], Sebastiano Giallongo [1], Paolo Fontana [4], Rosario Giuffrida [2], Giovanni Li Volti [2]

1. Division of Hematology, Department of General Surgery and Medical-Surgical Specialities, A.O.U. Policlinico Vittorio Emanuele, University of Catania, Catania, Italy.
2. Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy.
3. Division of Clinical Pathology, "Giovanni Paolo II" Hospital-A.S.P. Ragusa, Ragusa, Italy.
4. IOM Ricerca Srl, Viagrande, Italy.

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ABSTRACT

Multiple myeloma (MM) is a haematological disease characterized by plasma cells malignancy and extramedullary and/or peripheral blood progression with significant patient morbidity, thus representing an urgent clinical need. Besides multiple affected sites, resistance to therapy is strongly limiting current available therapies. The Wilms' tumour 1 (WT1) gene received particular attention in the last decades due to its role as oncogenic factors in acute myeloid leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia and myelodysplastic syndromes, thus becoming an attractive target for immune therapy in such dismal diseases. Herein, we aimed at reviewing current knowledge on the potential role of WT1 in MM pathogenesis and on its potential role as therapeutic target for MM.

1. Introduction

Multiple myeloma (MM) is a haematological tumor characterized by the accumulation of clonal, malignant plasma cells in the bone marrow. Plasma cell dyscrasias encompass a spectrum of diseases, which include asymptomatic premalignant phase (i.e. monoclonal gammopathy of unknown significance, MGUS), asymptomatic MM (i.e. smoldering MM, SMM) and malignant disease (i.e. MM and plasma cell leukemia) associated with end-organ damage and significant patient morbidity (1-3).
MM accounts for 10% of hematologic malignancies and 1% of all cancer deaths. Incidence increases with age (median 65 years), it is rare under 40, and twice as common in Afro-Caribbeans (4).
The only clearly defined epidemiological risk factor is radiation exposure.
Recent evidences support the hypothesis of a significant role of intercellular communication, milieu conditioning and soluble factors in several physiological and pathological conditions, including MM, with a particular focus on mesenchymal stem cells as main cellular component of bone marrow (5-20).

Several adverse prognostic factors including paraprotein levels, Hb count, serum calcium level, lytic bone lesions, and renal function have been identified. However, the parameters used do not address the fundamental genetic abnormalities of the disease and current approaches are strongly limited by resistance mechanisms (21-27). Deletion of 13q is an important adverse prognostic factor and the presence of hypodiploidy is strongly associated with poor prognosis in MM (28).
The Wilms' tumour 1 (WT1) gene was first identified in 1990 as a strong candidate predisposition gene for Wilms' tumour, which is a childhood renal neoplasm. Since then, a number of studies confirmed that WT1, which maps to chromosome 11p13, is mutated in the germline or somatically in~15% of Wilms' tumour cases (29).
Although WT1 gene was categorized at first as a tumor-suppressor gene, it was recently demonstrated that the wild-type WT1 gene holds a critical role during human development and acts as oncogenic rather than a tumor-suppressor function in many kinds of malignancies, including haematological malignancies such as acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia

* *Corresponding author*: Giovanni Li Volti, *livolti@unict.it*
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(CML) and myelodysplastic syndromes (MDS) (30-38). Its widespread expression in tumors has led WT1 to become one of the main target for cancer immune therapy (39, 40). However, there is very little data concerning the monitoring of WT1 levels in myeloma patients and its effect on prognosis. Here, we provide an overview of the WT1 gene, highlighting its role in MM pathogenesis.

2. WT1 structure and isoforms

The mammalian WT1 gene is ~50 kb in length, encoding proteins from as many as ten exons. There are at least 36 potential mammalian WT1 isoforms and all of them include four C2H2 Kruppel like zinc fingers similar to those found in the SP1 family of transcription factors. All non-mammalian vertebrates express only two isoforms, which differ by just three amino acids (lysine, threonine and serine; KTS). Although the functional relevance of the many WT1 isoforms is unclear, the importance of the +KTS and −KTS isoforms has been highlighted by the identification of splice site mutations in patients with Frasier syndrome (41). This pathology leads to male-to-female sex reversal and focal segmental glomerulosclerosis of the kidney. The dominant WT1 mutations create one allele that only produces the −KTS isoforms, leading to a reduced +KTS/−KTS isoform ratio. This suggests that both isoforms are essential and have different functions. A murine study demonstrated that mice lacking one of the + KTS or - KTS isoforms died neonatally due to incomplete renal development (42). In contrast, mice that specifically lack an insertion of 17 mammalian-specific amino acids encoded by an alternate exon 5 show no observable phenotype. Interestingly, the 17 amino acid domain acts as a transcriptional activator through interaction with the prostatic apoptosis response factor PAR4 (PAWR) and a mutation in this domain has been identified in a Wilms tumor, attesting to its importance at least in humans (43, 44).

3. Molecular mechanisms of WT1 action

Although several studies reported that WT1 functions as a transcription factor, some evidences support its post-transcriptional function, via RNA interactions. Of note, the data suggest that the –KTS isoforms may function mostly as transcriptional regulators, whereas the +KTS isoforms act predominantly post-transcriptionally. This suggests that the two isoforms perform identical or compensatory functions during these processes.

WT1 is a transcription factor that bind to the early growth response-1 DNA consensus sequence present in various growth factor gene promoters through its four zinc fingers. Of note, early cell-free studies showed that WT1, the −KTS isoforms in particular, binds to a consensus site, 5′-GCGGGGGCG-3′, which is identical to that found for EGR1, while +KTS isoform binds to a slightly different sequence (45, 46).

As previously reported, WT1 can act as either a transcriptional activator or repressor depending on the nature of the domain its binds. Furthermore, several post-translational modifications, including sumoylation and phosphorylation, have been shown to regulate WT1 subcellular localization and subsequently its function (47).

Rampal and co-workers demonstrated that WT1 can also regulate gene expression by modulating the epigenetic landscape, and this is relevant for disease progression (48). Loss-of-function WT1 mutations are observed in a subset of acute myeloid leukaemia (AML), as are mutations in the genes encoding TET family proteins, including IDH1/2 and TET2 which are involved in converting 5-methylcytosine (5mC) in the genome to 5-hydroxymethylcytosine (5hmC), often leading to gene expression increase. Accordingly, loss of function of WT1, TET2 or TET3 leads to a reduction in 5hmC and a similar impaired haematopoietic differentiation phenotype (49, 50).

The post-transcriptional function of WT1 was first observed by studying the + KTS isoform that specifically localizes and interacts with junction factors in kidney cells (51). Consistent with these observations, subsequent experiments showed that both major isoforms WT1, but not EGR1, can bind RNA derived from exon 2 of Igf2, the + KTS isoform showing greater affinity (52). It is possible to hypothesize that WT1 interacts with RNA-binding proteins in an isoform-dependent manner. These include the U2AF65 (U2AF2) and RBM4 junction factors, HNRPU (HNRNPU) and the Wilms tumor-associated protein WTAP, which is an essential component of the complex that performs RNA methylation and regulates splicing (53-56).

Furthermore, an interesting non-canonical function for WT1 in the regulation of mitotic checkpoints in cultured cell lines has also been demonstrated through direct interaction with the spindle assembly checkpoint protein MAD2 (57). It is clear that these observations could have implications for cancers that arise due to the mutation or dysregulated of WT1.

As previously reported, WT1 was initially shown to act as a transcriptional repressor, but to date the specific functions of WT1 in normal and neoplastic tissues remain to be fully elucidated. In normal ontogenesis, the WT1 gene is expressed in a time- and tissue-dependent manner, mainly in the foetal kidney, ovary, testis, and structures of mesodermal origin (58). Conversely, in adults, WT1 gene expression is limited to very few tissues, including kidney podocytes, stroma and splenic capsule, testicular Sertoli cells, ovarian granulosa cells, and CD34 positive hematopoietic progenitor stem cells (59-62).

Regarding malignant cells, most cases of acute leukemia and chronic myeloid leukemia blast crisis show abnormal expression of WT1 (63, 64). Of note, increased WT1 expression in acute leukemia is potentially used as a marker of minimal residual disease (MRD) (63). In this context, previous studies demonstrated that the level of WT1 expression in B lymphoma cells is significantly lower than that of acute leukemia (65); however, knowledge on the details of WT1 expression in MM and other types of mature B-cell neoplasms is poor.

4. WT1 in multiple myeloma

Most newly diagnosed cases of MM have a normal karyotype, and abnormalities are most often seen in advanced disease associated with increased proliferative activity of malignant cells (66).

In this context, the FISH test is required for specific chromosomal abnormalities detection in MM patients, allowing the identification of an abnormal clone in 45% of cases (67).

Deletions of 13q14, 17p13 and 11q23 are all associated with a poor prognostic factor, along with translocations of 14q32 involving chromosomes 4 and 16 have also been identified. Additional common cytogenetic alterations such as hyper or hypodiploidy and abnormalities involving chromosome 1q can be observed in MM patients. Such genetic instability is associated with altered proliferation or apoptosis pathways, including p53, the nuclear factor-kB pathway and RAS. Assays for the identification of susceptible MRD are essential for the detection of a relapse allowing the early initiation of immunotherapy interventions (68, 69). However, current MRD assays are heterogeneous and disease-specific approaches are sometimes required. Consequently, the idea of using WT1 as an MRD marker was an obvious area of study in myeloma patients.

Since the significance of the WT1 gene for multiple myeloma had not yet been clarified, Hatta et.al set out to determine clinical relevance by examining the association of clinical parameters and WT1 expression in the bone marrow in newly diagnosed MM patients (70). Expression of WT1 in myeloma was 59 to 1,600 copies / microg of RNA and 0.05 to 406.3 copies / microg of RNA / 100 plasma cells, respectively, lower than in leukemia. Interestingly, WT1 transcripts have been observed to increase as clinical factors worsen, including stage of the disease, Hb, protein M, platelet count, creatinine, urea nitrogen (BUN), serum alkaline phosphatase (ALP), beta2-microglobulin, calcium, activity of thymidine kinase (TK) and C-reactive protein (CRP). These results demonstrated that the expression level of WT1 could be an additional marker compared to the standard parameters considered in the risk assessment also for MM. In contrast with this, Saatci et.al, found no association between WT1 expression and multiple myeloma pathogenesis, although this difference may be partly explained by differences in sensitivity, use of qualitative versus quantitative analyses and the type of sample used (28). However, it was reported that WT1 expression levels in PB samples were significantly lower than in bone marrow samples in healthy individuals and the WT1 expression level was very low almost undetectable by quantitative RT-PCR. Consistent with this, Inou et al. reported that the expression of WT1 in BM was a log of magnitude greater than that of PB (71). Given the existence of low constitutive expression of WT1 in MM patients in peripheral blood, the analysis of WT1 expression may not be a practical genetic marker for routine clinical use, but the WT1 analysis in BM may be more sensitive PB in myeloma patients as suggested by Hatta's data.

Since WT1 expression appears to play an essential role in tumorigenesis and to be required to maintain the transformed phenotype and function, it has been suggested that the WT1 protein may be beneficial as a target antigen for cancer (72, 73). In this contest, CD8 positive cytotoxic T lymphocytes (CTLs) play a key role as effectors of antitumor immune responses, recognizing tumor-associated antigen-derived (TAA) peptides that are "processed" and presented on the surface of tumor cells in association with the major histocompatibility complex (MHC) class I molecules, leading to tumor cells' killing. Clinical evidence of antitumor immune responses efficacy has been obtained in several clinical settings, including the transplant versus leukemia (GVL) effect following allogeneic hematopoietic stem cell transplantation (HSCT)(74). These results strongly suggest that the WT1 protein could be a promising target antigen for cancer immunotherapy.

Since several types of normal cells, including haematopoietic progenitor cells, physiologically express WT1, knowing whether WT1-specific CTLs cause damage to normal tissue is critical. WT1-specific CTLs were shown to selectively kill leukemia cells but not normal hematopoietic progenitor cells, both of which express WT1. This can be explained by the difference in the level of WT1 expression between malignant and normal hematopoietic cells (65, 75).

It has been shown that many patients with haematological malignancies such as MDS, CML and AML produced WT1 IgM and IgG antibodies, indicating that not only WT1 responsive B cells in patients were activated but also the T cells needed to induce the class change of the WT1 antibody. Of note, the analysis in MDS patients revealed that the class change of the WT1 antibody from IgM to IgG occurred in conjunction with the progression of the disease from refractory anemia (RA) to refractory anemia with excess blasts (RAEB) with an increase in the amount of tumor that stimulates the patients' immune system (76).

Furthermore, the WT1 antibody disappeared after achieving complete remission in AML patients, suggesting that the decreased stimulation of the immune system by the leukemic cell-derived WT1 protein resulted in the interruption of antibody production (77).

Tyler et. al reported that MM cells are highly susceptible to perforin-mediated cytotoxicity by WT1-specific cytotoxic T lymphocytes (WT1-CTL)(78). Moreover, WT1 expression is sufficient to induce interferon-γ (IFN-γ) production by CTL. Isolated minimal clinical responses have been reported with WT1 peptide-based immunotherapy, which correlated with the expansion of functional WT1-CTL and their migration to the BM. The clinical significance of WT1-CTL has been demonstrated in patients with MM relapse and high-risk cytogenetics undergoing allogeneic T-cell-depleted hematopoietic stem cell transplantation (alloTCD-HSCT). MM patients received dose-escalating donor lymphocyte infusions (DLI) after transplantation, with increases in WT1-CTL frequencies in all serially monitored patients after alloTCD-HSCT and DLI. Interestingly, the emergence of WT1-CTL after DLI has been associated with the reduction or stabilization of specific MM markers. Clinically relevant disease responses were observed in MM patients who developed marked WT1-CTL responses. Furthermore, the co-expression of WT1 and CD138 observed in immunohistochemistry (IHC) in the BM samples of all MM patients tested proved that the expression of WT1 correlated with disease course (78).

In this context, Azuma et al. examined the mechanisms of cytotoxicity against WT1-specific CTL-mediated MM cells, focusing on the sensitivity of target cells to perforin-mediated cytotoxicity (79). Their data elucidate that WT1 expression levels in MM cells and lymphoma cells were both significantly lower than those in acute leukemia cells; however, MM cells were efficiently lysed by WT1-specific CTLs compared to lymphoma cells.

Furthermore, WT1-specific CTL precursors appeared to be present in MM patients but also in healthy individuals. Various types of tumor-associated antigens have recently been identified, however their distribution is not strictly limited to malignant cells and normal cells also express these antigens at a relatively low level (80-82).

The reason that only cancer cells and not normal cells are lysed by antigen-specific CTLs is associated with the tumor-associated antigen-derived peptide complex and the HLA molecule is expressed on normal cells at too low a level to be recognized by CTL.

However, a previous study revealed that a single major histocompatibility complex / peptide expressed on target cells can elicit a CTL response, but protective mechanisms are present in normal cells, although it is still unknown (83). Furthermore, CTLs are resistant to perforin-mediated cytotoxicity, as if they were sensitive they would be killed by the perforin they release. Therefore, elucidating the mechanism of resistance of CTLs to perforin-mediated cytotoxicity could provide an idea of the cause of the differential sensitivity to WT1-specific CTL-mediated cytotoxicity between myeloma cells and lymphoma cells (84).

5. Concluding remarks

Because the clinical outcomes of conventional chemotherapy for MM are not satisfactory, novel therapeutic approaches have been proposed, including cellular immunotherapy. Although some potential target antigens for immunotherapy of MM have been identified, the number of suitable target antigens recognized by CTLs directed against MM cells is still limited (85). The studies reported in this review may contribute to the development of novel immunotherapeutic strategies for MM and suggest that vaccination with a WT1-derived peptide or WT1-encoding DNA and adoptive immunotherapy using WT1-specific CTLs may provide an effective treatment option for MM as well as for acute leukemia.

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