The Wayne State University Waldenstrom’s Macroglobulinemia Preclinical Model for Waldenstrom’s Macroglobulinemia

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The Wayne State University Waldenstrom’s Macroglobulinemia xenograft model in mice with severe combined immune deficiency (WSU-WM-SCID) is the only preclinical animal model available for this disease. It is based on a permanent, EBV-IgM cell line (WSU-WM) established from a patient with a 10-year history of Waldenstrom’s macroglobulinemia (WM). These cells are CD5-CD10/CD19/CD20/CD22 and have t(8;14) (q24;32), t(12;17) (q24;q21), 2p-. WSU-WM cells also express DNA topoisomerase II (alpha and beta), and are bcl2/bclxL/bax-. Although the tumor has aggressive biological behavior with c-myc-IgH rearrangement, it has retained the salient features of WM. The breakpoint on 8q24 is downstream of c-myc exon 3, which is not usual for Burkitt-type breakpoints. WSU-WM cells also express both secretory (su) and membrane (mu) IgM mRNA and secrete IgM in culture supernatant. Histologically, WSU-WM-SCID xenograft tumors have lymphoplasmacytoid morphology. These features indicate biological, but not histological evolution. The WSU-WM-SCID is a model of a more aggressive and resistant WM usually seen toward the late stages of disease. It is, therefore, a particularly useful tool in developing new therapeutic strategies for the more aggressive WM, including targeted therapy, which exploits unique molecular characteristics of tumor cells.

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Because of the availability of well-characterized xenograft models in mice, and the relative ease of culture and handling, many studies are performed in this species. However, it is important to keep in mind that some of the molecular findings in mouse models may not be applicable to human disease due to differences in physiology and drug metabolism. This is particularly true in cases where genetic manipulation has been used to generate the models. For example, in the case of the Pim-1 transgenic mice, which have been extensively used to study the role of the Pim-1 kinase in tumorigenesis, it is not clear whether the findings in these models are relevant to human cancers.

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LIKE MANY OTHER indolent lymphoproliferative disorders in man, Waldenstrom’s macroglobulinemia (WM) is an incurable disease. However, the disease does respond to a variety of therapeutic agents with reduction of tumor burden and IgM, and relief of symptoms for variable lengths of time.1 Newer therapeutic agents have been added to the small list of “effective” agents against WM, like the nucleoside analogues, fludarabine and 2-chlorodeoxyadenosine (2CdA), and anti-CD20 monoclonal antibody, rituximab.2-4 Selecting such agents has remained largely empiric and is based on clinical trials in patients with WM.5 The use of “active” agents in combination also remains empiric and is guided primarily by evidence of some activity as single agents and non-overlapping toxicity profiles.

A preclinical animal model for WM can be used to investigate the efficacy of new therapeutic agents alone or in combination. Moreover, with the development and introduction of “targeted” therapy, a preclinical model provides a mechanism for validating binding/inhibition of the target and correlation with therapeutic efficacy. Preclinical efficacy trials (which are the animal counterpart of clinical trials in humans) are cheaper and faster to conduct and, more importantly, can avoid using ineffective or excessively toxic treatments in humans.

Similar to any experimental system, preclinical models of human disease have limitations. Investigators should, therefore, be cautious in drawing conclusions and especially in generalizing the observations and findings in such models.

In this report, we review the Wayne State University Waldenstrom’s Macroglobulinemia severe combined immunodeficiency (WSU-WM-SCID) mouse xenograft model for WM, which is the only preclinical animal model available for WM. Elucidation of its phenotypic, molecular, and genetic features should aid in its utility in the development of a more scientifically based, rational, and hopefully more effective therapy for WM.
Patient Characteristics

The patient from whom the WSU-WM cell line is derived was a 61-year-old man with a 10-year history of WM. At the time of establishing the cell line (year 10), the disease was progressive and unresponsive to standard therapy. Manifestations of disease aggressiveness included the development of malignant pleural effusion (from which the cell line was established), and the development, 3 months later, of meningeal lymphomatosis. Such manifestations are not common in WM unless there is a biologic evolution of the disease or morphologic transformation.

WSU-WM-SCID Mouse Xenograft Model

This is a subcutaneous tumor model developed by injecting human WSU-WM cells (in serum-free RPMI 1640) subcutaneously (SC) into the flank areas of 4-week-old SCID mice. Palpable tumors develop in about 2 weeks (Fig 1) that can then be propagated serially in vivo by transplanting tumor fragments into the flanks of a new batch of animals. CB17 and ICR SCID mice (Taconic Laboratories, Germantown, NY) have both been used with equal success. The success rate for in vivo propagation is 100% with more than 100 animals used for a variety of experiments. Although animals in initial experiments received one dose of cyclophosphamide prior to transplantation, such preconditioning is not necessary since the take rate was similar without it.

The SC model has advantages especially for preclinical efficacy trials. Easy access to tumor measurements allows for calculation of tumor growth inhibition (T/C), tumor growth delay (T-C), and Log_{10} kill, which are standard criteria for determining efficacy of a given therapeutic intervention. In addition to the SC tumors at local injection sites (flanks), animals developed lymphadenopathy but no hepatosplenomegaly. Histopathologic examination confirmed involvement of lymph nodes by WM. Flow cytometric examination of the bone marrow showed ~20% involvement by CD20^+ cells. Intravenous injection of WSU-WM cells has not been tried.

Morphological Features

WSU-WM cells growing in liquid culture have lymphoblastoid features with moderate amount of cytoplasm, large nucleus, and prominent nucleoli. These features are common to most, if not all, human lymphoid tumor cell lines growing in vitro. However, tissue sections of WSU-WM xenografts growing in SCID mice showed morphologic resemblance to WM. The growth consisted of diffuse proliferation of medium-sized plasmacytoid lymphocytes with fine chromatin pattern, prominent nucleoli, and small amounts of cytoplasm. These features indicate that the model is morphologically consistent with WM but has aggressive biological behavior that might be explained by the rearrangement of c-myc oncogene. WSU-WM cells secrete IgM in the culture supernatant and express both secretory (s^\#) and membrane (m^\#) components of the heavy chain IgM mRNA supporting its mature differentiated state.

Fig 1. Photograph of SCID mouse with bilateral SC tumors in the flank areas. The tumor on the left is larger (2.7 × 1.6 cm) compared with the one on the right (1.9 × 1.0 cm). These tumors were developed at the injection sites.
Phenotypic Characteristics

The WSU-WM model is that of CD5 - IgM WM. Cells also express CD20 (98%), CD19 (99%), CD10 (71%), and CD22 (20% to 30%). We have observed that WSU-WM xenografts tend to have higher expression of IgM and λ light chain (>90%) compared with WSU-WM cells in culture (20% to 30%).

Cytogenetic Characterization

WSU-WM cells have maintained stable karyotype as of the last characterization on September 18, 2001. Cells exhibited 46XY chromosomes and del(2)(p23), t(8;14)(q24;q32), t(12;17)(q24;q21) as clonal abnormalities.

Molecular-Genetic Characterization

Consistent with the cytogenetic finding of 8q24 breakpoint, WSU-WM cells showed rearrangement of one allele of the c-myc oncogene. The breakpoint on chromosome 8, however, is downstream of exon 3 of c-myc and is located between Xbal and HindIII restriction enzyme sites. This breakpoint is different from that usually seen in Burkitt's lymphoma, which is upstream of myc exon 1 or in intron 1. Cells were also EBV(-) (by EBV nuclear antigen stain), and have deleted κ and rearranged λ Ig genes. In addition, WSU-WM cells show high expression of topoisomerase II (alpha and beta) and low expression of topoisomerase I. Some apoptosis-regulating molecules were also expressed: bcl2 (high), bcl-xI (moderate). Bax, however, was undetected (Fig 2).

In Vitro Sensitivity of WSU-WM Cells to 2CdA

To demonstrate the utility of the WSU-WM cell line in pre-clinical investigation of therapeutic agents, some aspects of the nucleoside metabolism pathway investigation is presented here. There are two key cellular enzymes: deoxycytidine kinase (dCK) and 5'-nucleotidase (5'NT). The dCK phosphorylates 2CdA to its active metabolites (2CdAMP, 2CdADP, and 2CdATP) and 5'NT dephosphorylates the drug. The activity of these two enzymes in a given cell is one factor that determines sensitivity to the drug. Modulation of the level or activity of these enzymes by any biologic agent can also influence the sensitivity to 2CdA. WSU-WM cells express baseline activity of dCK and 5'NT conducive of phosphorylation (dCK 175 pmol/mg pr/min; 5'NT 11.0 pmol/mg pr/min) with a ratio of 15.9. There was significant accumulation of 2CdA metabolites within 3 hours of exposure to 1.0 μmol/L of 2CdA in culture within the cytosole and, more importantly, in DNA (Fig 3), supporting the enzyme data. 2CdA showed dose-dependent growth inhibition of WSU-WM cells with complete growth inhibition at 1.0 μmol/L (Fig 4). In vivo efficacy of 2CdA alone and in combination with other agents is being investigated in the WSU-WM SCID xenograft model.

DISCUSSION

The WSU-WM-SCID mouse xenograft model is the only pre-clinical animal model available for WM. It was established from a WM patient with aggressive, preterminal illness, where the disease had become progressive and resistant to conventional therapy. The aggressive clinical behavior manifested by the development of pleural effusion and meningeal lymphomatosis can be explained by the t(8;14) and c-myc rearrangement. This model, therefore, is that of aggressive WM rather than the
There are no consistent, recurrent chromosomal abnormalities for WM. A recent study suggests the WM cells lack immunoglobulin heavy chain locus translocations. However, there are other reports clearly describing such abnormalities. One way to explain this apparent discrepancy is to relate the cytogenetic findings to the clinical status of disease at the time of karyotyping. It is possible that IGH-myc rearrangement develops in a subset of WM patients, perhaps as a secondary event later in the course of disease. Such rearrangements will impart the aggressive behavior seen in some patients that can be rapidly fatal. The clinical course of disease in the patient from whom we established the WSU-WM line clearly changed during the last year to a more aggressive one. In support of such an interpretation are reports of certain chromosomal abnormalities seen at the time of disease progression and association of certain histologic features with aggressive clinical course. Unfortunately, we did not have the cytogenetic study done at an earlier point of the disease to determine if t(8;14) was a new event.

Histologically, the WSU-WM-SCID xenograft maintained features of WM with plasmacytoid lymphocytes. The disease, therefore, has evolved biologically but not histologically. From the practical standpoint, treatment of WM in its early phase is relatively simple since the disease is usually responsive to a variety of therapeutic agents. However, difficulty is encountered in the later phase when the disease becomes progressive and/or resistant. Our model represents the latter group.

With the development of targeted therapy, molecular and genetic features of a given tumor is becoming more important than its histopathology and may determine appropriate therapy in the future. For example, expression of certain surface antigens like CD20, CD22, and CD52 may determine responsiveness to monoclonal antibody therapy. Similarly, high expression of Bcl2, like in our model, may predict sensitivity to antisense bcl2 strategies. Modulation of such targets as DNA topoisomerases and apoptosis-regulating molecules might also guide the development of more rational therapy for cancer. The more detailed molecular

indolent, previously untreated, early diagnosis disease.

Fig 3. Time course and localization of 2CdAMP and 2CdATP in the cytosol and DNA of WSU-WM cell.

Fig 4. Dose-response effect of 2CdA on cell growth of WSU-WM cells in vitro.
characterization of our cell line should make it more useful as a model for developing new therapeutic strategies for WM.

REFERENCES