Malignant Lymphomas

Inhibition of the mammalian target of rapamycin and the induction of cell cycle arrest in mantle cell lymphoma cells

Mammalian target of rapamycin (mTOR) inhibitors represent a new class of potential anticancer agents. The mTOR inhibitor, rapamycin, inhibited proliferation in three mantle cell lymphoma (MCL) cell lines and reduced cyclin D3 expression while cyclin D1 levels remained unchanged. This finding was confirmed in cells from a MCL patient.

haematologica 2005; 90:1433-1434
(*http://www.haematologica.org/journal/2005/10/1433.html*)

Mantle cell lymphoma (MCL) is a distinct subtype of non-Hodgkin's lymphoma which is characterized by a poor response to conventional chemotherapy with a mean overall survival of approximately 3 to 4 years. Recently, therapeutic approaches including stem cell transplantation and monoclonal-antibody based therapies have improved response rates but only minor effects on overall survival have been observed.[1,2]

Therefore, new therapies are clearly needed for this still incurable disease. The hallmark of MCL is the translocation t(11;14), (q23, q32), which results in overexpression of cyclin D1 /Prad1. Although the precise role of cyclin D1 overexpression in the progression of MCL remains controversial, the presence of cyclin D1 overexpression or the t(11;14) is considered to be a highly specific marker for the diagnosis of this lymphoma.[3] In view of its altered cell cycle machinery, MCL seems to be an ideal candidate disease for targeted therapies directed at the cell cycle.

The mammalian target of rapamycin (mTOR) pathway has only recently been recognized as a target for cancer therapy. mTOR is emerging as a central controller of eukaryotic cell growth and proliferation, sensing mitogens in mammalian cells and allowing progression through the cell cycle. By activating downstream pathways, mTOR controls the translation of mRNA that encode proteins important for cell cycle progression, including cyclin D1.[4] Indeed, mTOR inhibition resulted in cell cycle arrest in a variety of cellular models[5,6] and is considered to represent a promising new class of cytostatic anticancer agents.[4]

In our current study, we analyzed the effect of mTOR inhibition on cell cycle progression in MCL cell lines carrying the translocation t(11; 14)(q23, q32). Culture conditions and experimental procedures including proliferation assays, separation methods and Western blots were as previously described .[7] The cell lines Granta 519, NCEB-1 and Jeko-1 were kindly provided by Dr. Mark Raffeld, National Cancer Institute, NIH, Bethesda, USA.

Treatment with rapamycin inhibited proliferation of Granta and NCEB cells. This was associated with an accumulation of cells in the G1 phase of the cell cycle in both MCL cell lines (Figure 1A, 1B). As reported for other cell types, apoptosis was not induced by treatment with rapamycin (*data not shown*).[5] Cyclin D3 expression was strongly reduced in both cell lines by treatment with rapamycin while cyclin D1 expression was not significantly changed (Figure 1C). This interesting finding was confirmed in one additional MCL (Jeko-1) cell line (*data not shown*) and primary MCL cells (Figure 2) suggesting that cyclin D1 is not a target of mTOR inhibitors in MCL. In line with these data, we and others have identified cyclin D3 to be an important target of rapamycin in malignant and normal lymphocytes.[5,8] It is very important from a clinical point of view that plasma concentrations of more than 15 ng/mL are easily achievable in patients treated with rapamycin.[9]

In addition to cyclin D3 expression, cyclin A expression was strongly reduced in Rapamycin-treated NCEB and primary MCL cells but was only slightly downregulated in

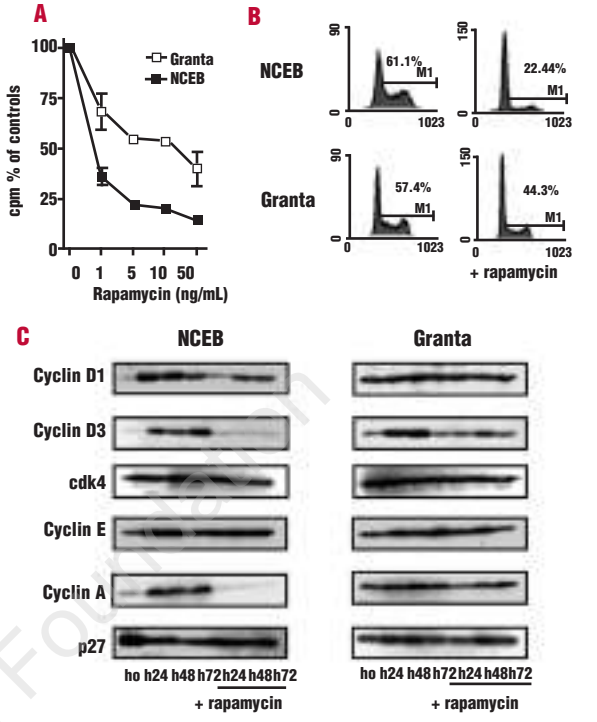

Figure 1. Rapamycin induces cell cycle arrest in MCL cell lines. **A.** Cells were cultured in medium containing 20% fetal calf serum and rapamycin at the indicated concentrations. ^{3}H thymidine incorporation was measured after 2 days of culture in triplicate. One representative experiment out of three performed is shown. Data (mean±SEM) are presented as % proliferation [(thymidine incorporation in the presence of Rapamycin) (thymidine incorporation in medium alone)*100)]. **B.** To analyze cell cycle distribution, NCEB and Granta cells were cultured with or without rapamycin. Propidium iodide-staining was performed after 48 hours of culture. The percentage of cells in the S/G2/M phase of the cell cycle is given. These tests were repeated once with very similar results. **C.** To synchronize cells in the cell cycle better, Granta and NCEB cells were cultured for 36 hours in the absence of fetal calf serum. Afterwards they were cultured for up to 72 hours in medium containing 20% fetal calf serum with or without rapamycin 10 ng/mL. Cyclin, cdk and p27 expression was analyzed by immunoblotting with specific antibodies in total cell lysates (100 µg). Protein concentrations were normalized by the Bio-Rad assay method. Two additional experiments gave very similar results.

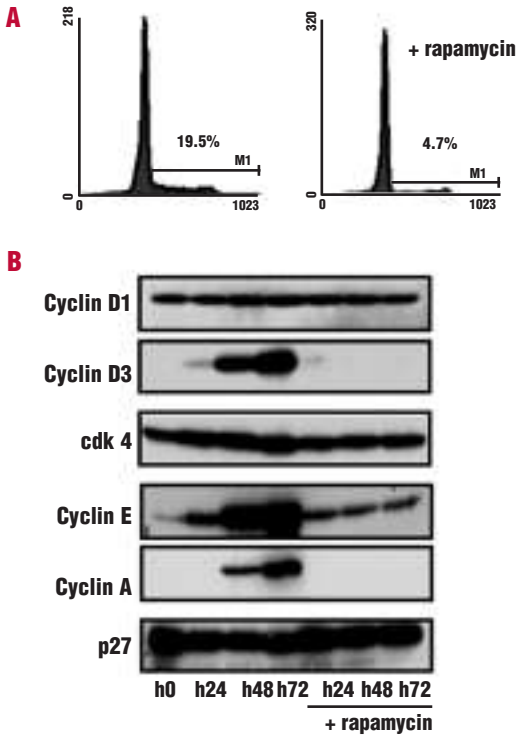

Figure 2. The effect of rapamycin on primary MCL cells. Propidium iodide-staining (A) was performed after 72 hours of culture of purified MCL cells in the presence of the immunostimulatory oligonucleotide DSP30 and interleukin-2. Rapamycin was added from the beginning of the culture as indicated. (B): cyclin, cdk and p27 expression was analyzed by immunoblotting with specific antibodies after the indicated times. Protein concentrations were normalized by the Bio-Rad assay method.

Granta cells. This corresponded to a strong reduction in cdk2 activity in NCEB and to a lesser extent in Granta cells (*data not shown*). In contrast to our findings in both cell lines, cyclin E expression was reduced in rapamycin-treated primary MCL cells (Figure 2), making cyclin E an additional target in primary MCL. In this regard, the effect of rapamycin on primary MCL cells closely resembles the effect on activated B-cell chronic lymphocytic leukemia cells.[5] Two mTOR inhibitors, RAD001 and CCI-779 are currently undergoing clinical development for hematologic malignancies.[10] Interestingly, preliminary data confirming single agent activity in relapsed MCL patients have been reported in MCL patients.[11] Extensive safety data exist for RAD001 which is approved in Europe as an immunosuppressive agent in solid organ transplantation.

Taken together, our data demonstrate that inhibition of mTOR results in cell cycle arrest in MCL cells by affecting expression of critical cell cycle regulatory molecules, including cyclin D3, cyclin E and cyclin A, while cyclin D1 is not affected. Our promising results in MCL model cell lines as well as in freshly isolated MCL cells are strong arguments for further evaluation of mTOR inhibitors in this still incurable disease. Our future work will deal with potential strategies to combine these cytostatic agents with cytotoxic or targeted therapies to improve therapeutic efficacy.

Susanne Hipp, Ingo Ringshausen,* Madlene Oelsner,* Christian Bogner,* Christian Peschel,* Thomas Decker°*

**IIIrd Department of Medicine, Technical University of Munich, Munich, Germany; °Schwerpunktpraxis für Tumortherapie, Weingarten, Germany*

Funding: this work was supported by a research grant from the Technical University of Munich (KKF H30-97) and a grant from the Deutsche Forschungsgemeinschaft DE 771.

Key words: MCL, rapamycin, RAD001, cyclin D1, cyclin D3.

Correspondence: Thomas Decker, M.D., IIIrd Department of Medicine, Technical University of Munich, Ismaninger Str. 15, 81675 Munich. Phone: international +49.89.41404110. Fax: international +49.89.41404879. E-mail: t.decker@lrz.tu-muenchen.de

References

1. Howard OM, Gribben JG, Neuberg DS, Grossbard M, Poor C, Janicek MJ, et al. Rituximab and CHOP induction therapy for newly diagnosed mantle-cell lymphoma: molecular complete responses are not predictive of progression-free survival. J Clin Oncol 2002;20:1288-94.
2. Freedman AS, Neuberg D, Gribben JG, Mauch P, Soiffer RJ, Fisher DC, et al. High-dose chemoradiotherapy and anti-B-cell monoclonal antibody-purged autologous bone marrow transplantation in mantle-cell lymphoma: no evidence for long-term remission. J Clin Oncol 1998;16:13-8.
3. Bertoni F, Zucca E, Cotter FE. Molecular basis of mantle cell lymphoma. Br J Haematol 2004;124:130-40.
4. Bjornsti MA, Houghton PJ. The tor pathway: a target for cancer therapy. Nat Rev Cancer 2004;4:335-48.
5. Decker T, Hipp S, Ringshausen I, Bogner C, Oelsner M, Schneller F, et al. Rapamycin-induced G1 arrest in cycling B-CLL cells is associated with reduced expression of cyclin D3, cyclin E, cyclin A, and survivin. Blood 2003;101:278-85.
6. Boffa DJ, Luan F, Thomas D, Yang H, Sharma VK, Lagman M, et al. Rapamycin inhibits the growth and metastatic progression of non-small cell lung cancer. Clin Cancer Res 2004; 10:293-300.
7. Bogner C, Ringshausen I, Schneller F, Fend F, Quintanilla-Martinez L, Hacker G, et al. Inhibition of the proteasome induces cell cycle arrest and apoptosis in mantle cell lymphoma cells. Br J Haematol 2003;122:260-8.
8. Hleb M, Murphy S, Wagner EF, Hanna NN, Sharma N, Park J, et al. Evidence for cyclin D3 as a novel target of rapamycin in human T lymphocytes. J Biol Chem 2004;279:31948-55.
9. Groth CG, Backman L, Morales JM, Calne R, Kreis H, Lang P, et al. Sirolismus (rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Sirolismus European Renal Transplant Study Group. Transplantation 1999;67:1036-42.
10. Huang S, Houghton PJ. Targeting mTOR signaling for cancer therapy. Curr Opin Pharmacol 2003;3:371-7.
11. Witzig TE, Geyer SM, Ghobrial I, Inwards DJ, Fonseca R, Kurtin P, et al. Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. J Clin Oncol 2005; 23:5347-56.