Prof. dr n. med. Mariusz A. Wąsik – informacja biograficzna

Studia i kształcenie podyplomowe	stopień	okres	dziedzina studiów
Akademia Medyczna, Wrocław	M.D.	1973–1979 1979–1980	medycyna (staż podyplomowy)
Harvard Medical School and Boston University	–	1985–1987	immunologia
Dana-Farber Cancer Institute/Harvard Medical School	–	1987–1988	immunologia
Mallory Institute of Pathology, Boston, MA Beth Israel Hospital/Harvard Medical School	– –	1988–1991 1991–1993	patologia hematopatologia

STANOWISKA I ZATRUDNIENIE

1980–1983	asystent, Instytut Immunologii i Terapii Doświadczalnej i. L. Hirszfelda PAN, Wrocław, Polska
1983–1985	Research Assistant Professor, Department of Microbiology, State University of New York, Buffalo, NY Department of Pathology & Laboratory Medicine, University of Pennsylvania Medical Center, Philadelphia, PA:
1993–2001	Assistant Professor & Associate Director of Hematology Laboratory
2001–2006	Associate Professor
2001–	Director of Experimental Hematology
2005–	Director of Hematopathology
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INFORMACJE UZUPEŁNIAJĄCE

Członkostwo w towarzystwach i organizacjach naukowych: UPenn Cancer Center (1995–), UPenn Graduate Group in Immunol (1997–), Cephalon Sci Adv Board (2004–2011), Lymphoma Res Found Panel of Sci Consult (2008–).
Recenzent czasopism naukowych: EMBO J, J Invest Dermatol, Blood, Cancer, J Leuko Biol, Am J Path, Am J Clin Path, Leukemia, Cancer Res, Proc Natl Acad Sci USA, FEBS Lett, J Clin Invest, Lancet Oncol, New Engl J Med, Science, Nature Med, Mol Cancer Ther, PLOS One.
Członkostwo w zespołach eksperckich: NIH Narodowy Instytut Zdrowia – the Tumor Cell Biology Study Section (2003–2011), SPORE grant review panels (2003–), Program Project Grant review panels (2006–), Special Emphasis Panel (2012–), Provocative Questions Initiative (2013–)

WYRÓŻNIENIA I NAGRODY

NCI Shannon Award (1999), Chair of UPenn Cancer Center Lymphoma Symposia 2000, 2002, and 2005, invited speaker at national and international symposia (2000–).

PUBLIKACJE: ogółem 165 (154 oryginalne i 11 przeglądowych) z liczbą ponad 6000 cytowań; http://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/47420164/.

ZAINTERESOWANIA I OPIS BADAŃ WŁASNYCH

A. Personal Statement. I am involved in investigating the oncogenic role of aberrant cell signaling and epigenetic gene silencing in lymphoid malignancies to understand better the pathogenesis of lymphomas and other malignancies, develop new diagnostic and monitoring tools, and novel therapies targeting the aberrantly activated signaling pathways and activating the epigenetically silenced tumor suppressor genes. Our group has focused on the following pathways and genes (*please see also Contribution to Science, part V*):

1. **Aberrant signaling in B-cell lymphomas**. Our original studies identified mTOR as therapeutic target in a subtype of B-cell lymphoma called post-transplant lymphoproliferative disorder and, for that matter, human malignancy of any kind. Our extensive follow up studies showed that the highly specific rapamycin-related inhibitors of the enzymatic complex containing mTOR (mTORC1) display a strong growth-suppressive effect on the whole spectrum of T- and B-cell lymphomas including the large B-cell lymphoma. We found that the mechanism of mTORC1 activation is lymphoma-type dependent and involves the key primary oncogenic signals capable of activating simultaneously, and the various degree, cell signaling pathways/kinases that are upstream of mTORC1: PI3K/AKT, MEK/ERK, and other including Syk kinase. Furthermore, we found that combination of rapamycin-type mTORC1 inhibitor with an inhibitor of MNK1/2, two related kinases able to phosphorylate eIF4E augmenting its mTORC1-induced activity, triggers apoptotic cell death of lymphoma cells. Our current efforts focus on further characterization of the mechanisms of mTORC1 activation, foremost the role of Bruton tyrosine kinase (BTK) active in mantle cell lymphoma (MCL) and other B-cell malignancies in this process and, in collaboration with Jerry Glickson's group, development of the imaging-based methods of in vivo visualization of BTK inhibition, and analysis of the BTK-dependent genes and proteins with focus on cell metabolism. Recently, we have also succeeded in demonstrating that the combination of chimeric antigen receptor T cells against CD19 (CART19 cells) with an inhibition of BTK by a small compound Ibrutinib is highly effective against MCL in pre-clinical *in vitro* and *in vivo* models.

1. Majewski M., Korecka M., Kossev P., Li S., Goldman J., Moore J., Silberstein L., Nowell P.C., Schuler W., Shaw L., **Wasik M.A.**: Immunosuppressive macrolide SDZ RAD inhibits growth of human EBV-transformed B lymphocytes in vitro and in vivo; a potential approach to prevention and treatment of posttransplant lymphoproliferative disorders (PTLDs). *Proc. Natl. Acad. Sci. USA*, 97: 4285–4290, 2000. (PMID 10759564)
2. Majewski M., Korecka M., Joergensen J., Fields L., Kossev P., Schuler W., Shaw L., **Wasik M.A.**: Immunosuppressive TOR kinase inhibitor everolimus (RAD) suppresses growth of cells derived from posttransplant lymphoproliferative disorder at allograft-protecting doses. *Transplantation*, 75:1710–1717, 2003. (PMID 12777861)
3. Wlodarski P., Kasprzycka M., Liu X., Marzec M., Slupianek A., **Wasik M.A.**: Activation of mTOR in transformed B lymphocytes is nutrient-dependent but independent of Akt, MEK, IGF-I, and serum. *Cancer Res.*, 65: 7800–7808, 2005. (PMID 16140948)
4. Ruella M., Kenderian S.S., Shestova O., Fraietta J.A., Qayyum S., Zhang Q., Maus M.V., Liu X., Nunez-Cruz S., Klichinsky M., Kawalekar O.U., Milone M., Lacey S.F., Mato A., Schuster S.J., Kalos M., June C.H., Gill S., **Wasik M.A.**: The Addition of the BTK Inhibitor Ibrutinib to Anti-CD19 Chimeric Antigen Receptor T Cells (CART19) Improves Responses against Mantle Cell Lymphoma. *Clin Cancer Res*. 2016 Jan 27. [Epub ahead of print]. (PMID 26819453)

2. **Mechanisms of malignant cell transformation by the chimeric NPM/ALK kinase.** Through our own and collaborative efforts, we found that pathways involving mTORC1, STAT3, PI3K/AKT, MEK/ERK, and STAT5b are constitutively activated by NPM/ALK. Studying the effect of signaling proteins on the epigenetic gene silencing and other cell functions are the main focus of our ongoing investigation. SHP-1, STAT5a, and IL-2Rg emerged from these studies as key epigenetically silenced tumor suppressors that act by targeting NPM/ALK. We also found that NPM/ALK-induced PD-L1 and ICOS foster immune evasion and the NPM/ALK induced expression of HIF1a controls metabolism of the malignant cells, adding to the ever-growing list of diverse cell functions regulated by NPM/ALK. Perhaps not surprisingly, we have shown that transfected NPM/ALK is capable of transforming normal CD4+ T lymphocytes, the "natural" target of this oncogenic kinase. Finally, we found that NPM/ALK succeeds in transforming the target CD4+ T cells by "high jacking" the cell signaling pathways physiologically activated in these target cells by the cytokines from the IL-2 family, the key regulators of maturation and activation of the normal T cells. These studies provide insight into complexities of the mechanisms of kinase-induced oncogenesis, in general, including the key role of aberrant signal transduction and ensuing gene expression induction and silencing in the process of malignant cell transformation.

3. **Role IL-2R-type signaling in malignant T-cell transformation**. We found that cutaneous T-cell lymphoma (CTCL) displays activation of IL-2R-associated Jak/STAT pathway that is transient in the early stage and constitutive in the late stage of the lymphoma. The constitutive Jak/STAT activation is due, at least in part, to the lack of expression of SHP-1 phosphatase. Importantly, this work identified the mechanism underlying lack of SHP-1 expression as hypermethylation of the CpG DNA sequences within the SHP-1 promoter. Furthermore, we implicated STAT3 as well as members of the epigenetic gene silencing machinery including DNA methyltransferase DNMT1 into silencing of the SHP-1 gene. STAT3 acts by both fostering the silencing at the SHP-1 gene promoter level and promoting DNMT1 expression at both gene and transcript levels. We have also found that the early stage cutaneous T-cell lymphoma cells express the immunosuppressive factor FoxP3 and that this expression is induced by the STAT5a/b transcription factor complex controlled by the cytokines such as IL-2 and IL-15. We have also found expression of the immunosuppressive receptor: ligand pair PD-1 and PD-L1. PD-1 expression is strong in the atypical cells of early stages of CTCL (patch and plaque) and gradually diminished in the cells at the tumor and, in particular, large-cell transformation stage. In contrast to PD-1 (and FoxP3), PDL-L1 is expressed at all stages of CTCL by atypical lymphocytes and seems particularly strong in the large transformed cells, suggesting that while all three proteins contribute to immunosuppression in the early disease stage, PD-L1 expression seems key to the immunosuppression at its late stages. Finally, we have found that CTCL cells and tissues ubiquitously express another immunosuppressive cell-surface protein CD80 (B7-1), the key ligand of CD152 (CTLA-4). CD80 expression in CTCL cells is induced by STAT5a and STAT5b through their joint ability to transcriptionally activate the CD80 gene. Hence, we identified a new mechanism of immune evasion in CTCL suggesting that the CD80-CD152 axis may become a therapeutic target in this type of lymphoma.

4. **Aberrant gene expression in lymphomas**. Over the last 15 years, we been applying genome scale methods, at first by DNA oligonucleotide arrays and, more recently RNA sequence analysis, to identify aberrantly expressed genes and to, subsequently, study contributions of their protein products to malignant cell transformation. Our early studies (Lee et al. Am J Path 2000)

focused on the differential gene expression related to the progression in advanced T-cell lymphoma involving skin. Later studies examined the genes regulated by IL-2, IL-15, and IL-21 in CTCL, NPM/ALK and STAT3 in the lymphomas expressing the kinase, DNA methyltransferases in the T-cell lymphomas and mTORC1 in B-cell lymphomas (diffuse large cell and mantle cell lymphomas). Most recently, we have added DNA sequencing to identify also genomic structural abnormalities in malignant cells currently focusing on mantle cell lymphoma.

The key five findings and related papers

I. Identification of the mechanistic link between an oncogene and epigenetic silencing of tumor suppressor genes. We have shown that an oncogenic kinase NPM-ALK kinase uses a transcription factor STAT3 to inhibit expression of tumor suppressor genes by at least three different mechanisms. First, STAT3 induces expression of DNA methyltransferase DNMT1. Second, STAT3 enhances DNMT1 expression by inhibiting expression of miR-21, which selectively inhibits expression of DNMT1 mRNA. Finally, STAT3 anchors DNMT1, other DNMTs and at least one protein deacetylase: HDAC1 to gene promoters of three different tumor suppressors: SHP-1 phosphatase, STAT5a, and IL-2Rg. Expression of any of these proteins inhibits expression of NPM-ALK validating them as *bona fide* tumor suppressors.

1. Zhang Q., Wang H.Y., Woetmann A., Raghunath P.N., Odum N., **Wasik M.A.**: STAT3 induces transcription of the DNA methyltransferase 1 (DNMT1) gene in malignant T-lymphocytes. *Blood*, 108: 1058–1064, 2006. (PMID 16861352)
2. Zhang Q., Wang H.Y., Marzec M., Raghunath P.N., Nagasawa T., **Wasik M.A.**: STAT3- and DNA methyltransferase 1-mediated epigenetic silencing of SHP-1 tyrosine phosphatase tumor suppressor gene in malignant T lymphocytes. *Proc Natl Acad Sci USA*, 102:6948–6953, 2005. (PMID 15870198)
3. Zhang Q., Wang H.Y., Liu X., **Wasik M.A.**: Stat5a is epigenetically silenced in the NPM/ALK-transformed T lymphocytes and acts in such cells as tumor suppressor by inhibiting expression of the NPM/ALK oncogene. *Nature Med*., 13: 1341–1348, 2007. (PMID 17922009)
4. Zhang Q., Wang H.Y., Liu X., Bhutani G., Kantekure K., Raghunath P.N., **Wasik M.A.**: IL-2R common g chain is epigenetically silenced by nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) and acts as a tumor suppressor by targeting NPM-ALK. *Proc. Natl. Acad. Sci. USA*, 108: 11977–11982, 2011. (PMID 21715655)

II. Identification of the mechanistic link between an oncogene and tumor immune evasion in cancer. While it has been well known that cancer cells escape immune destruction, the mechanism by which malignant cells acquire this ability has not been clear. We have shown that a highly oncogenic NPM-ALK kinase by constitutively activating STAT3 transcription factor induces persistent expression of both soluble (IL-10 and TGFß) and cell membrane (PD-L1) inhibitory molecules. We have also shown that NPM-ALK induces via STAT3 expression of ICOS a growth factor for ALK+ lymphoma cells but, arguably, inhibitor of immune response by absorbing ICOS-L. Finally, we have shown that cutaneous T-cell lymphoma cells utilize the constitutively activated STAT5 to induce expression of a different immunosuppressive cell-membrane protein CD80 providing an another direct link between oncogenesis and tumor immune evasion.

1. Kasprzycka M., Marzec M., Liu X., Zhang Q., **Wasik M.A.**: NPM/ALK oncoprotein induces the T regulatory cell phenotype by activating STAT3. *Proc. Natl. Acad. Sci. USA*, 103: 9964–9969, 2006. (PMID 16766651)

2. Marzec M., Zhang Q., Goradia A., Raghunath P.N., Liu X., Paessler M., Wang H.Y., Wysocka M., Cheng M., Ruggeri B., **Wasik M.A.**: Oncogenic kinase NPM/ALK induces through STAT3 expression of immunosuppressive protein CD274 (PD-LI, B7-H1). *Proc. Natl. Acad. Sci. USA*, 105: 20852–20857, 2008. (PMID 19088198)
3. Zhang Q., Wang H.Y., Kantekure K., Paterson J.C., Liu X., Schaffer A., Paulos C., Milone M.C., Odum N., Turner S., Marafioti T., **Wasik M.A.**: Oncogenic kinase NPM/ALK induces expression of the cell-growth stimulatory receptor ICOS. *Blood*, 118: 3062–3071, 2011. (PMID 21765024)
4. Zhang Q., Wang H.Y., Wei F., Liu X., Paterson J.C., Roy D., Mihova D., Woetmann A., Ptasznik A., Odum N., Schuster S.J., Marafioti T., Riley J.L., **Wasik M.A.**: Cutaneous T cell lymphoma expresses immunosuppressive CD80 (B7-1) cell surface protein in a STAT5-dependent manner. *J Immunol* 192: 2913–2919, 2014. (PMID 24523507)

III. **Development of *in vitro* model of oncogenesis**. We have shown that lentiviral vector-induced expression of NPM-ALK in normal human CD4+ T lymphocytes (the "natural" target of NPM-ALK) resulted in their malignant transformation *in vitro*. The transformed cells became immortalized and display morphology and immunophenotype of patient-derived ALK+ lymphomas. The perpetual cell growth, activation of the key signal transduction pathways and expression of CD30, IL-10 and PD-L1/CD274 are strictly dependent on NPM-ALK activity and expression. Implantation of the NPM-ALK transformed CD4+ T lymphocytes into immunodeficient mice resulted in formation of tumors indistinguishable from the parental cells and patient-derived ALK+ lymphomas. This study demonstrated for the first time ever an effective malignant transformation *in vitro* of normal human cells by a single oncogene. This method of cell transformation should permit to study the early stages of carcinogenesis, mechanisms of malignant progression, and the effects of early therapeutic intervention. We have also found that NPM-ALK transforms the target CD4+ T cells by "high jacking" signaling pathways physiologically used by cytokines from the IL-2 family.

1. Zhang Q., Wei F., Wang H.Y., Liu X., Roy D., Xiong Q.B., Jiang S., Medvec A., Danet-Desnoyers G., Watt C., Tomczak E., Kalos M., Riley J.L., **Wasik M.A.**: A potent oncogene NPM-ALK mediates malignant transformation of normal human CD4+ T lymphocytes. *Am J Path* 183: 1971–1980, 2013. (PMID 24404580).
2. Marzec M., Halasa K., Liu X., Wang H.Y., Cheng M., Baldwin D., Tobias J.W., Schuster S.J., Woetmann A., Zhang Q., Turner S.D., Ødum N., **Wasik M.A.**: Malignant transformation of CD4+ T lymphocytes mediated by oncogenic kinase NPM/ALK recapitulates IL-2-induced cell signaling and gene expression reprogramming. *J Immunol* 191: 6200–6207, 2013. (PMID 24218456)

IV. **Identification and characterization of mTOR as therapeutic target in human lymphoma and cancer in general**. *The details are provided above in the Personal Statement.* The key relevant publications are:

1. Marzec M., Liu X., Kasprzycka M., Witkiewicz A., Raghunath P., El-Salem M., Robertson E., Odum N., **Wasik M.A.**: IL-2- and IL-15-induced activation of the rapamycin-sensitive mTORC1 pathway in malignant CD4+ T lymphocytes. *Blood*, 111: 2181–2189, 2008. (PMID 18025151)
2. El-Salem M., Raghunath P.N., Marzec M., Liu X., Kasprzycka M., Robertson E., **Wasik M.A.**: Activation of mTORC1 signaling pathway in AIDS-related lymphomas (ARL). *Am. J. Pathol.*, 175: 817–824, 2009. (PMID 19608873)

3. Marzec M., Liu X., Wysocka M., Rook A.H., Odum N., **Wasik M.A.**: Simultaneous inhibition of mTOR-containing complex 1 (mTORC1) and MNK kinase induces apoptosis of cutaneous T-cell lymphoma (CTCL) cells. *PLoS One*, 6: e24849, 2011. (PMID 21949767)
4. Lee S.C., Marzec M., Liu X., Wehrli S., Kantekure K., Ragunath P.N., Delikatny E.J., Glickson J.D., **Wasik M.A.**: Decreased lactate concentration and glycolytic enzyme expression reflect inhibition of mTOR signal transduction pathway in B-cell lymphoma. *NMR in Biomed*., 26: 106–114, 2013. (PMID 22711601)

V. **Examination of Cyclin D1/CDK4 kinase complex for its oncogenic properties and as therapeutic target in mantle cell lymphoma (MCL)**. Because chromosomal translocation t(11;14) resulting in the ectopic expression of Cyclin D1 is critical for pathogenesis of MCL through association of the Cyclin D1 protein with the CDK4/6 kinases and, consequently, activation of the cell cycle, we examined sensitivity of MCL cells to the highly selective CDK4/6 inhibitor. We have found that MCL cells predominantly express activated CDK4 and that the inhibitor suppresses their growth. This was the first evidence that targeted therapy may prove effective in lymphoma of any kind. Our pre-clinical findings have eventually been validated in a clinical trial with MCL patients (Leonard et al. Blood, 2012). We have also determined the Cyclin D1 isoform profile in MCL and, in collaborative studies, we have also shown that Cyclin D1 requires nuclear localization to act as an oncogene, is inhibited by Fbx4 and cooperates with PRMT5.

1. Marzec M., Kasprzycka M., Lai R., Gladden A.B., Wlodarski P., Tomczak E., Nowell P., Deprimo S.E., Sadis S., Eck S., Schuster S.J., Diehl J.A., **Wasik M.A.**: Mantle cell lymphoma cells express predominantly cyclin D1a isoform and are highly sensitive to selective inhibition of CDK4 kinase activity. *Blood*, 108: 1744–1750, 2006. (PMID 16690963)
2. Gladden A.B., Woolery R., Aggarwal P., **Wasik M.A.**: Diehl JA: Expression of constitutively nuclear cyclin D1 in murine lymphocytes induces B-cell lymphoma. *Oncogene*, 25: 998–1007, 2006. (PMID 16247460)
3. Vaites L.P., Lee E.K., Lian Z., Barbash O., Roy D., **Wasik M.**, Klein-Szanto A.J., Rustgi A.K., Diehl J.A.: The Fbx4 tumor suppressor regulates cyclin D1 accumulation and prevents neoplastic transformation. *Mol. Cell. Biol.*, 31:4513–23, 2011. (PMID 21911473)
4. Li Y., Chitnis N., Nakagawa H., Kita Y., Natsugoe S., Yang Y., Li Z., **Wasik M.**, Klein-Szanto A.J., Rustgi A.K., Diehl J.A.: PRMT5 is required for lymphomagenesis triggered by multiple oncogenic drivers. *Cancer Discov*., 5: 288–303, 2015. (PMID 25582697)