CURRICULUM VITAE

Part I. General Information

Date prepared: December, 2008

Name Elzbieta Kaczmarek

Office Address Beth Israel Deaconess Medical Center
RN 370E
99 Brookline Avenue
Boston, MA 02215
Tel: 617 667 1677

E-mail: ekaczmar@bidmc.harvard.edu **FAX:** 617 632 0838

Place of Birth: Warsaw, Poland

Education:

1974-1978	MS	Warsaw University, Warsaw, Poland
1978-82		Postgraduate Studies: Institute of Nuclear Research, Warsaw, Poland
1985	Ph.D.	Jagiellonian University, Cracow, Poland

Research Fellowships:

1982	Visiting Research Fellow	Biochemistry	Max Plank Institute, Munich, Germany
1883-85	Research Fellow	Biochemistry	Institute of Nuclear Research, Warsaw, Poland

Postdoctoral Training:

1985-88	Research Fellow	Biochemistry	Beth Israel Hospital, Boston, MA

Academic Appointments:

1989-94	Instructor	Harvard Medical School, Boston, MA
1995-2002	Instructor	Harvard Medical School, Boston, MA
2002-present	Assistant Professor	Harvard Medical School, Boston, MA

Hospital Appointments:

1989-94	Research Associate	Beth Israel Hospital, Boston, MA
1995-present	Associate Scientist Ph.D.	Beth Israel Deaconess Medical Center, Boston, MA

Professional Societies:

1992-94 International Society of Thrombosis and Haemostasis

	Member
1993-94	International Society for Fibrinolysis and Thrombolysis Member

Awards and Honors:

1985	Polish Ministry of Health Award for the Ph.D. thesis

Part II: Research, Teaching, and Clinical Contributions

A. Narrative report of research.

Most of my time and effort is spent on research. This also includes teaching and supervising of postdoctoral fellows, technicians, students and less experienced researchers.

For the last 6 years my research has been focused on the P2 receptor signaling, primarily in endothelial cells (EC), but recently also in other cell types, including hepatocytes, skeletal muscle and pancreatic beta-cells.

Our initial data indicated that EC stimulated with extracellular nucleotides, responded with increased intracellular calcium levels and phosphorylation of several proteins, including FAK, paxillin, $p130^{cas}$, ERK and Akt. We associated these signaling pathways with various physiologic EC functions, such as cell spreading, migration, proliferation and apoptosis. Our results showed that in EC extracellular nucleotides induced cytoskeletal rearrangements, significantly increased cell migration, and affected EC proliferation, suggesting the involvement of P2 receptors in angiogenesis. This hypothesis was supported by the recent findings showing a connection between P2Y receptors and $\alpha_v\beta_3(\beta_5)$ integrins, whose role in angiogenesis has already been documented.

Our recent study indicated that extracellular nucleotides, via P2Y receptors and calcium/calmodulin-dependent kinase kinase (CaMKK), mediated activation of AMP-activated kinase (AMPK), recognized as a key energy regulator and a primary protective kinase in diabetes mellitus.

We also showed that extracellular nucleotides activated endothelial nitric oxide synthase (eNOS), an enzyme that generates nitric oxide (NO), whose role in preventing atherosclerosis and its complications is well established. NO maintains vascular tone, exerts anti-inflammatory effects through inhibition of NF-κB activation, protects EC from apoptosis by reducing oxidative damage and inhibits smooth muscle cell (SMC) proliferation and thus development of neointimal hyperplasia. Impaired NO production leads to EC dysfunction and accelerated atherosclerosis in patients with diabetes mellitus. We identified a new nucleotide-induced signaling pathway of eNOS activation in EC, that is calcium and PKCδ-dependent but does not require the activities of AMPK, PI3K/Akt and ERK. Importantly, this new pathway was functional in EC cultured under high glucose conditions, suggesting that extracellular nucleotides can override the damaging effects of high concentrations of glucose, such as diabetic vasculopathy, by maintaining NO bioavailability. We validated these data *in vivo*, by showing increased phosphorylation of eNOS in hearts of mice treated with ATP.

Moreover, we observed up-regulation of expression of anti-apoptotic A1 and anti-apoptotic and anti-inflammatory A20 factors at the mRNA and protein levels in EC treated with extracellular nucleotides.

These combined effects of extracellular nucleotides (activation of AMPK and eNOS, and upregulation of A1and A20) strongly suggest that the use of P2 receptor agonists may represent a novel therapeutic approach for blocking endothelial dysfunction and atherogenic mechanisms, and protecting the vasculature from diabetic complications.

These data provided the basis for two new grant applications.

Our long-term plan is to investigate purinergic mechanisms in EC in the context of inflammatory responses, wound healing, and angiogenesis under normal and high glucose conditions.

We also collaborate with laboratories at BIDMC/Harvard Medical School (Drs. C. Ferran and L. Otterbein), Boston University School of Medicine (Drs. JK Blusztajn and V. Trinkaus-Randall), and Warsaw Medical University (Drs. Gaciong and Koziak).

B. Research Funding Information

Completed and Ongoing Research Support

1997-2000	NIH/RO1 (HL57307)	Co-PI	Ecto-ADPases in transplantation
2000-2001	Phyllis and Paul Fireman Fellowship BIDMC/HMS	PI	Determination of purinergic signaling in endothelial cells leading to up-regulation of E-selectin expression
2002-2006	NIH/R01 (HL066167)	PI	Purinergic signaling in endothelial cells
2006-2010	NIH/R01 (HL080130)	Invest	Vascular remodeling in transplant atheriosclerosis
2007-2008	JDRF (5-2007-736)	PI	Protection of endothelial cell dysfunction by P2 receptor signaling

C. Current Research Activities

Project	Role
Role of extracellular nucleotide-induced increase in the intracellular ATP levels in endothelial cell proliferation and apoptosis.	PI
Role of extracellular nucleotides and P2 receptor signaling in regulation of A1 and A20 expression and eNOS activity in endothelial cell cultured under normal and high glucose.	PI
Heterogeneity of P2 receptor-mediated calcium responses in EC.	PI
Role of extracellular nucleotides in ROS formation in endothelial cells exposed to high glucose.	PI
Evaluation of the atheroprotective potential of P2 receptor agonists in the diabetic ApoE-null mice.	PI
Mechanism of A20 expression regulation in endothelial and smooth muscle cells under high glucose.	Investigator
Mechanism of A20-induced regulation of eNOS expression in endothelial cells.	Investigator

D. Teaching experience

1. Local contributions

Local Presentations

2002 Beth Israel Deaconess Medical Center, Harvard Medical School
Lecture at the Transplant Center Seminars
Title: Purinergic signaling in endothelial cells
Attendings, Principal Investigators, post-doctoral fellows, graduate students affiliated with the Transplant Center

2003 Beth Israel Deaconess Medical Center, Harvard Medical School
Lecture at the Transplant Center Seminars
Title: Extracellular nucleotides and endothelial cell migration
Attendings, Principal Investigators, post-doctoral fellows, graduate students affiliated with the Transplant Center

2004 Beth Israel Deaconess Medical Center, Harvard Medical School
Lecture at the Gastroenterology Seminars
Title: Purinergic signaling in endothelial cells
Attendings, Principal Investigators, post-doctoral fellows, graduate students affiliated with the Gastroenterology Department

2005 Beth Israel Deaconess Medical Center, Harvard Medical School
Lecture at the Surgery Center Seminars
Title: Extracellular nucleotides, cellular energy levels and possible consequences.
Attendings, Principal Investigators, post-doctoral fellows, graduate students affiliated with the Surgery Department

2006 Beth Israel Deaconess Medical Center, Harvard Medical School
Lecture at the Transplant Center Seminars
Title: Extracellular nucleotide-induced activation of AMPK in EC
Attendings, Principal Investigators, post-doctoral fellows, graduate students affiliated with the Surgery Department

Advisees and trainees

Year	Name	Current Position
1995, 1996	Matthew Boes	Resident in Surgery, USA
1995-98	Katarzyna Koziak	Senior scientist, Poland
1996	Katarzyna Wloka	Postdoctoral fellow, USA
1999	Kaja Malanowska	Scientist, Poland
2000	Seo-Kiat Goh	Resident in Medicine, Thailand
2002-2004	Feng Chen	Ph.D. student, USA
2002-2004	Marcia Wink	Scientist, Brazil
2002-2004	Robert Jarzyna	Scientist, Poland
2004	Jena Harb	Graduate student, USA
2004	Fabio Fonseca	Postdoctoral fellow, USA
2005	Anke Specht	Graduate student, Germany
2005-2008	Cleide Goncalves da Silva	Postdoctoral fellow, USA
2006	Anne Spriestersbach	Graduate student, Germany
2006-2008	Elizabeth Maccariello	Clinician, Brazil
2008	Carl Geahchan	Medical School Student, Lebanon

2. Regional, National or International Contributions

2006 Session Chair at the 8th International Symposium on Adenosine and Adenine nucleotides, Ferrara, Italy. Session: Basic and Applied Pharmacology

Part III. Bibliography

Original Articles

1. **Kaczmarek E**. Interaction between human prothrombin and staphylocoagulase from Staphylococcus aureus Newman D2. Acta Hematol Polon 1985;16:144-50.
2. **Kaczmarek E**, Kaminski M, McDonagh J. Fibrinogen-Sepharose interaction with prothrombin, prethrombin 1, prethrombin 2, and thrombin. Biochim Biophys Acta 1987;914:275-82.
3 **Kaczmarek E**, McDonagh J. Thrombin binding to the Aα-, Bβ-, and γ-chains of fibrinogen and to their remnants contained in fragment E. J Biol Chem 1988;263:13896-900.
4. Lee MH, **Kaczmarek E**, Chin DT, Oda A, McIntosh S, Bauer KA, Clyne LP, McDonagh J. Fibrinogen Ledyard (Aα Arg_{16}—>Cys): biochemical and physiologic characterization. Blood 1991;78:1744-52.
5. **Kaczmarek E**, Lee MH, McDonagh J. Initial interaction between fibrin(ogen) and tissue plasminogen activator (t-PA): The Gly-Pro-Arg-Pro binding site on fibrin(ogen) is important for t-PA activity. J Biol Chem 1993;268:2474-9.
6. Bovill EG, McDonagh J, Triplett DA, Arkin CF, Brandt JT, Hayes TE, **Kaczmarek E**, Long T, Rock WA. Performance characteristics of fibrinogen assays. Arch Pathol Lab Med 1993;117:58-66.
7. Margossian SS, Slayter HS, **Kaczmarek E**, McDonagh J. Physical characterization of recombinant tissue plasminogen activator. Biochim Biophys Acta 1993;1163:250-6.
8. **Kaczmarek E**, Liu Y, Berse B, Chen C, McDonagh J. Biosynthesis of plasma factor XIII: evidence for transcription and translation in hepatoma cells. Biochim Biophys Acta 1995;1247:127-34.
9. **Kaczmarek E**, Koziak K, Sevigny J, Siegel JB, Beaudoin AD, Bach FH, Robson SC. Identification and characterization of vascular ecto-ATP diphosphohydrolase/CD39. J Biol Chem 1996;271:33116-22.
10. **Kaczmarek E**, Koyamada N, Miyatake T, Robson SC. Vascular ATP diphosphohydrolase and xenotransplantation. Xeno 1996;4:98-101.
11. Robson SC, **Kaczmarek E**, Koziak K, Siegel JB, Millan M, Candinas D, Bach FH. Loss of ATP diphosphohydrolase following endothelial cell activation. J Exp Med 1997;185:1-12.
12. Kopp C, Geczy C, Siegel JB, Winkler H, **Kaczmarek E**, Bach FH, Robson SC. Molecular incompatibility between porcine TFPI and human factor Xa. Transplantation 1997;63:749-58.
13. Kopp C, Robson SC, Siegel JB, Anrather J, Winkler H, Grey S, **Kaczmarek E**, Bach FH and Geczy C. Regulation of monocyte tissue factor activity by allogeneic and xenogeneic endothelial cells. Thromb Haemost 1998;79:529-38.
14. Von Albertini M, Palmetshofer A, **Kaczmarek E**, Koziak K, Stroka D, Grey S, Stuhlmeier KM, Robson SC. Extracellular ATP and ADP activate transcription factor NF-κB and induce endothelial cell apoptosis. Biochem Biophys Res Commun 1998;248:822-9.

15. Soares MP, Amuniapp A, **Kaczmarek E**, Koziak K, Wrighton C, Steinhauslin F, Ferran C, Winkler H, Bach FH, Anrather J. Overexpression of a transdominant negative mutant of p65/RelA inhibits NF-κB without sensitizing to apoptosis. J Immunol 1998;161:4572-8.

16. Schulte am Esch II J, Sevigny J, **Kaczmarek E**, Siegel JB, Imai M, Koziak K, Robson SC. Structural and biochemical characteristics of CD39 ATP diphosphohydrolase. Biochemistry 1999;38:2248-58.

17. Kittel A, **Kaczmarek E**, Sevigny J, Lengyel K, Csizmadia E, Robson SC. CD39 as a caveolar-associated ectonucleotidase. Biochem Biophys Res Commun 1999;262:596-9.

18. Imai M, **Kaczmarek E**, Koziak K, Sevigny J, Goepfert C, Guckelborger O, Scizmadia E, Shulte am Esch II J, Robson SC. Suppression of ATP diphosphohydrolase/CD39 in human vascular endothelial cells. Biochemistry 1999;38:13473-9.

19. Koziak K, Sevigny J, Robson SC, Siegel JB, **Kaczmarek E**. Analysis of CD39/ATPD diphosphohydrolase (ATPDase) expression in endothelial cells, platelets and leukocytes. Thromb Haemost 1999;82:1538-44.

20. Koziak K*, **Kaczmarek E***, Kittel A, Sevigny J, Blusztajn JK, Shulte am Esch II Jr, Guckelborger O, Goepfert C, Qawi I, Robson SC. Palmitoylation targets CD39/endothelial ATP diphosphohydrolase to caveolae. J Biol Chem 2000; 275:2057-62. (*** - joint first authors**)

21. Imai M, Goepfert C, **Kaczmarek E**, Robson SC. CD39 modulates IL-1 release from activated endothelial cells. Biochem Biophys Res Comm 2000;270:272-8.

22. Imai M, Takigami K, Guckelberger O, **Kaczmarek E**, Csizmadia E, Bach FH, Robson SC. Recombinant adenoviral-mediated CD39 gene transfer prolongs cardiac xenograft survival. Transplantation 2000;70:864-70.

23. Goepfert C, Imai M, Brouard S, Csizmadia E, **Kaczmarek E**, Robson SC. Vascular NTPDase modulates endothelial cell activation and apoptosis. Molecular Medicine 2000;6:591-603.

24. Koziak K, **Kaczmarek E**, Park SY, Fu Y, Avraham S, Avraham H. RAFTK/Pyk2 involvement in platelet activation is mediated by phosphoinositide 3-kinase. Brit J Haematol 2001;114:134-40.

25. Sass G, Soares MC, Yamashita K, Seyfried S, Zimmermann WH, Eschenhagen T, **Kaczmarek E**, Ritter T, Volk HD, Tiegs G. Heme oxygenase-1 and its reaction product, carbon monoxide, prevent inflammation-related apoptotic liver damage in mice. Hepatology. 2003;38:909-18.

26. Berberat PO, Katori M, **Kaczmarek E**, Anselmo D, Lassman C, Ke B, Shen X, Busuttil RW, Yamashita K, Csizmadia E, Tyagi S, Otterbein LE, Brouard S, Tobiasch E, Bach FH, Kupiec-Weglinski JW, Soares MP. Heavy chain ferritin acts as an antiapoptotic gene that protects livers from ischemia reperfusion injury. FASEB J. 2003;17:1724-6.

27. Knosalla C, Giovino MA, Harper D, **Kaczmarek E**, Gollackner B, Cooper DK, Robson SC. Relative effects of GAL and GAL porcine hematopoietic cells on primate platelet aggregation and endothelial cell activation: implications for the induction of mixed hematopoietic chimerism in the pig-to-primate model. Xenotransplantation.

2004;11:72-7

28. Guckelberger O, Sun XF, Sevigny J, Imai M, **Kaczmarek E**, Enjyoji K, Kruskal JB, Robson SC. Beneficial effects of CD39/ecto-nucleoside triphosphate diphosphohydrolase-1 in murine intestinal ischemia-reperfusion injury. J Thromb Haemost. 2004;91:576-86.

29. Dwyer KM, Robson SC, Nandurkar HH, Campbell DJ, Gock H, Murray-Segal LJ, Fisicaro N, Mysore TB, **Kaczmarek E**, Cowan PJ, d'Apice AJ. Thromboregulatory manifestations in human CD39 transgenic mice and the implications for thrombotic disease and transplantation.J Clin Invest. 2004;113:1440-6.

30. Gollackner B, Goh SK, Qawi I, Buhler L, Knosalla C, Daniel S, **Kaczmarek E**, Awwad M, Cooper DK, Robson SC. Acute vascular rejection of xenografts: roles of natural and elicited xenoreactive antibodies in activation of vascular endothelial cells and induction of procoagulant activity.Transplantation. 2004;77:1735-41.

31. Gollackner B, Qawi I, Daniel S, **Kaczmarek E**, Cooper DK, Robson SC. Potential target molecules on pig kidneys recognized by naive and elicited baboon antibodies. Xenotransplantation. 2004;11:380-1.

32. Klepeis VE, Weinger I, **Kaczmarek E**, Trinkaus-Randall V. P2Y receptors play a critical role in epithelial cell-cell communication and migration. J Cell Biochem 2004; 93:1115-33.

33. Sass G, Seyfried S, Soares MP, Yamashita K, **Kaczmarek E**, Neuhuber WL, Tiegs G. Cooperative effect of biliverdin and carbon monoxide on survival of mice in immune-mediated liver injury. Hepatology, 2004;40:1128-35.

34. **Kaczmarek E**. Erb L, Koziak K, Jarzyna R, Wink MR, Guckelberger O, Blusztajn JK, Trinkaus-Randall V, Weisman GA, Robson SC. Modulation of endothelial cell migration by extracellular nucleotides. Involvement of focal adhesion kinase and phosphatidylinositol 3-kinase-mediated pathways. Thromb Haemost. 2005;93:735-42.

35. Wu Y, Sun X, **Kaczmarek E**, Bianchi E, Usheva A, Robson SC. RanBPM associates with CD39 and modulates ecto-nucleotidase activity. Biochem J. 2006;396:23-30.

36. Silva C, Jarzyna R, Specht A, **Kaczmarek E**. Extracellular nucleotides and adenosine independently activate AMP-activated protein kinase in endothelial cells. Involvement of P2 receptors and adenosine transporters. Circ Res. 2006;98:e39-e47.

37. Künzli BM, Berberat PO, Giese T, Csizmadia E, **Kaczmarek E**, Baker C, Halaceli I, Büchler MW, Friess H, Robson SC. Upregulation of CD39/NTPDases and P2 receptors in human pancreatic disease. Am J Physiol 2007;292:G223-30.

38. Koziak K, Bojakowska M, Robson S, Bojakowski K, Religa P, Soin J, Gaciong Z, **Kaczmarek E**. Overexpression of CD39/nucleoside triphosphate diphosphohydrolases-1 prevents restenosis after angioplasty. J Thromb Haemost. 2008;6:1191-7.

39. Silva C, Specht A, Wegiel B, Ferran C, **Kaczmarek E**. Mechanism of purinergic activation of endothelial nitric oxide synthase in endothelial cells. Circulation 2009;119:871-9.

40. Xu C, Shmukler BE, Nishimura K, **Kaczmarek E**, Rossetti S, Harris PC, Wandinger-Ness A, Robert L. Bacallao RL, Alper SL. Attenuated, flow-induced ATP release contributes to absence of flow-sensitive, purinergic Ca^{2+} signaling in human ADPKD cyst epithelial cells. Am J Physiol Renal Physiol. 2009 Feb 25 (Epub ahead of print).

41. Wegiel B, Baty CJ, Csizmadia E, Scott JR, Gallo D, Ardavan Akhavan A, Beek Y. Chin BY, **Kaczmarek E**, Zuckerbraun BS, Alam J, Bach FH, Otterbein LE. Cell surface biliverdin reductase regulates innate immunity in response to endotoxin. Blood (paper under review).

Proceedings of meetings:

1. **Kaczmarek E**, Kurecki T, Henschen A. Staphylocoagulase: purification and interaction with human prothrombin. (1985) In: Jeljaszewicz J, editor. The Staphylococci. Zbl Bakt Suppl. 14. Gustav Fisher Verlag, Stuttgart, 1985, p. 353-4.

2. **Kaczmarek E**, Siegel JB, Sevigny J, Koziak K, Hancock WW, Beaudoin A, Bach FH, Robson SC. Vascular ATP diphosphohydrolase (CD39/ATPDase). In: Plesner L, Kirley TL and Knowles AF, editors. Ecto-ATPases. Proceedings of First International Workshop on Ecto-ATPases. Plenum. New York. 1997, p. 171-85.

3. Robson SC, Imai M, Sevigny J, Guckelberger O, Schulte Am Esch J, Gaciong Z, **Kaczmarek E**, Rosenberg RD, Enjyoji K. Perturbations in hemostasis and thrombosis in cd39-mutant mice: implications for experimental therapeutics of CD39/NTPDase 1 In: Vanduffel L and Lemmens R, editors. Ecto-ATPases. Proceedings of the Second International Workshop on Ecto-ATPases and Related Ectonucleotidases. Shaker Publishing B.V., Maastricht, The Netherlands 1999, p. 209-19.

4. Imai M, Takigami K, Guckelberger O, Lin Y, Sevigny J, **Kaczmarek E**, Goepfert C, Enjyoji K, Bach FH, Rosenberg RD, Robson SC. CD39/vascular ATP diphosphohydrolase modulates xenograft survival. Transplantation Proceedings 2000;32:1.

Review articles:

Kaczmarek E. Staphylococcal coagulase. Progr Hyg Exptl Med 1986;40:268-88.

Books:

McDonagh J, **Kaczmarek E**, Lee MH. Fibrinogen and factor XIII: biology and disorders of fibrin formation and cross-linking. In: Handin RI, Lux SE, Stossel TP, editors. Blood. Principles and Practice of Hematology. JB Lippincott, 1995, p.1219-59.

Extracellular ATP and adenosine as the regulators of endothelial cell function. Springer. The author of one chapter and the editor of the book (May 2009).