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Advances in Purine and Pyrimidine Metabolism in Health and Diseases

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Summary

In June, 2015, the Purine and Pyrimidine Society (PPS) organized the 16th biennial symposium on Purine and Pyrimidine metabolism at the Faculty House of Columbia University, New York City. This exciting meeting focused on these important molecules, new developments in inborn errors of metabolism; therapeutic analogs. In addition, the biochemistry of mammalian and non-mammalian systems were discussed. Due to significant advances in molecular medicine, the boundaries between clinical and basic sciences have merged into exciting translational research, of which a small portion was highlighted in the pre-symposium.

Keywords

Purines; pyrimidines; antimetabolites; inborn errors

Introduction

The Purine and Pyrimidine Society (PPS) organized the 16th biennial symposium on Purine and Pyrimidine metabolism at the Faculty House of Columbia University, New York City in June, 2015 (PP15). This symposium series was initiated as an international meeting on human purine metabolism in Tel Aviv, Israel in 1973 with expansion of the focus to include pyrimidine metabolism (1985) and convened every three years. Proceedings of the last meetings were published in *Nucleosides, Nucleotides, and Nucleic Acids*.[1–6] Although sponsorship of the international symposia had traditionally been local and without a sustaining organizing body, a European Society for the Study of Purine and Pyrimidine Metabolism in Man (ESSPPMM) was formed in 1987 and subsequently met every two years, in some years, in conjunction with the international symposium. At the 2003 joint international and European symposium in Egmond aan Zee, The Netherlands, an agreement was reached to merge the international and European groups in a new organization called the Purine and Pyrimidine Society. This decision was formally approved in 2005 by the ESSPPM membership at their final meeting in Prague, Czech Republic. Because rapid advances in the field dictate frequent updates, PPS decided to sponsor biennial symposia, the first of which (called PP07) was held in Chicago, USA, in 2007. Subsequent meetings were held in Stockholm, Sweden (2009), Tokyo, Japan (2011), and Madrid, Spain (2013). The

latest symposium, PP15, enabled basic, clinical, and translational investigators to present their novel research and via active discussions, interact to share thoughts about molecular and clinical mechanisms of purine and pyrimidine metabolism in health and in diseases.

The disorders covered included abnormalities of uric acid metabolism, gout, cancer, Lesch-Nyhan disease, immunological disorders, mitochondrial diseases, and other rare inborn errors of metabolism. In addition, fundamental studies on nucleoside transporters and receptors, purine and pyrimidine enzyme regulation and analogs, as well as non-mammalian metabolism were actively discussed. The presentations spanned a wide-array of investigations including: clinical characterization of patients, next-generation sequencing and genome-wide association studies of rare and common disorders, basic enzymology and molecular structural analyses, cellular and animal models, and translational combinations of research techniques.

Scientific Sessions

PP15 was comprised of a pre-symposium followed by ten scientific sessions (Table 1) with 21 invited speakers and 27 oral presentations selected from the abstract submissions. A highlight of the meeting was the Anne Simmonds Memorial Lecture presented by Dr. Michael Becker (University of Chicago)[7]. In addition, 50 excellent posters were presented primarily by young investigators with three selected as PPS Poster Award winners (Table 2).

Disorders of Purine and Pyrimidine Metabolism

The conference began with a pre-symposium session focused on translational research of purine and pyrimidine dysmetabolism. A dysfunction of the purine salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) was the first inborn error of purine metabolism to be associated with human conditions, gout and Lesch-Nyhan syndrome.; therefore, it was fitting to initiate the meeting with Dr. Rosa Torres's presentation on the pathogenesis of severe HGPRT deficiency, which is still incompletely understood. Using a human neuronal culture model of Lesch-Nyhan syndrome, NT2/D1 cells treated with hypoxanthine, Dr. Torres observed alterations in transcriptional factors including WNT4 and engrailed homeobox 1 required for neuronal development as well as transcripts for dopamine, adenosine, and serotonin receptors. Dr. Lena Rasmussen reported alterations of mitochondria including abnormalities of mitochondrial enzyme activities, deoxynucleoside triphosphate levels, and DNA in peripheral blood mononuclear cells that correlated with cognitive decline in adults. Dr. Kimiyoshi Ichida reported exciting data linking two genetic variants in the ATP-binding cassette transporter sub-family G, member 2 (ATPG2) efflux transporter of urate and other molecules to increased risks for overproduction and underexcretion forms of hyperuricemia. Finally, Dr. André van Kuilenburg gave an elegant overview of the biochemistry and pathophysiology of purine and pyrimidine metabolism, which now total more than 30 individual disorders.

In the first session devoted to gout and hyperuricemia and chaired by Drs. Tappei Takada and Michael Becker, Dr. Takada provided evidence that rapidly progressive hyperuricemia was more strongly associated with dysfunctional variants in *ABCG2* than with established

environmental risk factors. Dr. Shunya Uchida reported retrospective data, analyzed by a propensity score method, linking elevation of serum uric acid to severity of chronic kidney disease. Using genome-wide association studies, Dr. Hirotaka Matsuo identified five gout susceptibility loci. These loci included known urate transporter genes (*ABCG2* and *SLC2A9*) as well as two novel gout loci (*MYL2-CUX2* associated with cholesterol and diabetes mellitus and *DNIH-2* encoding a glutamate signal regulator). In an interesting clinical study, Dr. Juan Puig reported a sensitive ultrasound technique to detect microtophi in gout patients without clinical tophi.

The second session, chaired by Drs. Monika Löffler and Juan Puig, was devoted to inborn errors of purine and pyrimidine metabolism. Dr. Löffler provided an update on two disorders of pyrimidine de novo synthesis: orotic aciduria due to UMP synthase mutations and Miller syndrome caused by dihydroorotate dehydrogenase mutations. Dr. Ivan Sebesta reported data on detection of hereditary xanthinuria in the Czech population using a diagnostic algorithm with: a) measurements of serum and urine uric acid, b) assessment of urinary xanthine, and c) allopurinol loading test. Dr. Puig, on behalf of Dr. Gisbert de la Cuadra, described two patients with Lesch-Nyhan disease that caused such severe self-injurious behavior that total dental extractions were necessary to improve the qualities of their lives. Dr. Kiyoko Kaneko described studies of uricase-knockout mice that showed increased urinary excretion of uric acid and decreased excretion of 8-OH deoxyguanosine, a marker of oxidative stress, suggesting that uric acid is an anti-oxidant.

Drs. Godefridus Peters and Lars Petter Jordheim chaired the third session, which highlighted purines and pyrimidines in cancer. Dr. Peters provided an update on chemotherapy protocols used to optimize efficacy of the uracil analog 5-fluorouracil (5FU) and described a relationship between genome alterations and efficacy, as well as the use of proteomics to reveal novel changes in protein expression after 5FU administration to patients. Dr. Jordheim reported that decreased activity of 5’-nucleotidase cN-II in tumor cells enhanced tumor growth in mice. In cultured human breast cancer cells (MDA-MB-231), decreased cN-II was associated with increased proliferation and survival under low-glucose conditions suggesting enhanced bioenergetic metabolism. Dr. van Kuilenburg identified high expression of uridine-cytidine kinase (UCK) 2 in neuroblastoma raising the possibility that analogs that need UCK2 for activation have therapeutic potential for this tumor type.

For nucleosides to cross cell membranes, membrane transport proteins are required. Drs. Imogen Coe and Marçal Pastor-Anglada chaired the fourth session that concentrated on purine and pyrimidine transporters. The SLC28 and SLC29 transporter families direct uni- or bi-directional flow of nucleosides and nucleoside-analog drugs. Dr. Coe described the SLC29 family (equilibrative nucleoside transporters [ENTs]) that modulate purine nucleoside flux and contribute to purinergic signaling, cellular homeostasis, and clinical efficacy of drugs. Dr. Pastor-Anglada focused on the SLC28 gene family (human Concentrative Nucleoside Transporter proteins: hCNT1, hCNT2, and hCNT3) that mediate Na^+-coupled cellular uptake of nucleosides thereby contributing to nucleoside-derived drug pharmacokinetics. Dr. Aránzazu Mediero reported that the purine nucleoside analog ticagrelor, a known $P2Y_{12}$ antagonist and inhibitor of cellular adenosine uptake via ENT1, can inhibit osteoclast differentiation in vitro via the blockade of adenosine uptake. Dr. Alex

Bicket provided evidence that calcium regulates ENT1 function in HEK293 and RT4 cells via an interaction between ENT1 and calmodulin, a calcium signaling transducer. Dr. Masayuki Sakiyama described functional alterations associated with the I269T variant of the human sodium-dependent phosphate cotransporter 1 (NPT1) of urate; in a *Xenopus* oocyte model, this NPT1 variant was found to increase Vmax, but not the Km, of urate transport thereby providing a mechanism for its association with renal underexcretion gout.

The next session, chaired by Drs. Staffan Eriksson and Stefan Lutz, highlighted regulation of purine and pyrimidine pathway enzymes. Dr. Eriksson reviewed key enzymes in the de novo synthesis pathways, which includes two cytosolic enzymes thymidine kinase 1 (TK1) and deoxycytidine kinase (dCK) and two mitochondrial enzymes thymidine kinase 2 (TK2) and deoxyguanosine kinase (dGK). His recent work has elucidated the reaction mechanism and negative cooperativity of TK2 as well as regulation of TK1-like enzymes via phosphorylation and oligomeric forms. Dr. Lutz described innovative development of transgenes of engineered nucleoside kinases that can phosphorylate isotopically labeled nucleoside analogs, which enable positron emission tomography (PET) imaging of combinations of kinases with specific nucleoside analogs in cells. Dr. Jordheim reported the kinetics of fludarabine on inhibition of 5’-nucleotidase cN-II indicating a mechanism for this purine nucleoside analog in cancer cells.

Drs. Bruce Cronstein and Wajahat Mehal co-chaired the session on purines and pyrimidines in inflammation and inflammatory diseases. Dr. Cronstein highlighted the role of adenosine, acting on the A2A receptor, in promoting wound healing and anti-fibrotic action of A2A receptor antagonists, such as tenofovir, in hepatic and dermal inflammatory states. Dr. Mehal focused on the mechanisms by which adenosine enhances assembly and activation of the inflammasome; his data indicate that adenosine up-regulation of HIF-1α induces sustained production of IL-1β and a prolonged inflammatory response. Dr. Jordheim reported a physical interaction between cN-II and the inflammasome protein NLRCA/Ipaf, which may modulate cell viability. Dr. Coriulo described striking osteoarthritis in A2A receptor knock out mice indicating the importance of local adenosine release in maintaining cartilage homeostasis by preventing inflammation.

Defects of mitochondrial purine and pyrimidine metabolism have brought new attention to these critical pathways. Drs. Liya Wang and Michio Hirano co-chaired the seventh session devoted to this subject. Dr. Wang reviewed the expanding number of human genetic diseases due to mutations that disrupt mitochondrial deoxynucleoside triphosphate (dNTP) pool homeostasis or mitochondrial DNA (mtDNA) replication machinery, which, in turn, lead to mtDNA instability manifesting as mtDNA depletion, multiple deletions, or both. The causative mutations affect purine and pyrimidine salvage pathway genes (*TK2*, *DGUOK*), de novo synthesis (*RRM2B*), nucleoside catabolism (*TYMP*), and mtDNA replication (*POLG, POLG2*, and *C10orf2*). Dr. Hirano provided an update on mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), which is due to loss of function *TYMP* mutations, which cause severe thymidine phosphorylase deficiency leading to toxic accumulations of thymidine, that, in turn, cause dNTP pool imbalances and mtDNA instability, which is amenable to enzyme replacement therapies via hematopoetic stem cell transplantation. Dr. Caterina Garone described the clinical spectrum of TK2 deficiency and

striking therapeutic effects of deoxythymidine and deoxycytidine monophosphates (dTMP +dCMP) molecular bypass therapy in the Tk2 H126N knock in mouse model. Dr. Chiara Rampazzo reported a new regulatory protein, deoxyribonucleoside triphosphohydrolase (SAMHD1) in the maintenance of mitochondrial dGTP pools. Dr. Yolanda Cámara reported that administration of deoxynucleosides in vitro to POLG-deficient cells increases mitochondrial dNTP pools and ameliorates the mtDNA replication defect, which has clinical therapeutic implications. Dr. Claus Desler described alterations in the dNTP pools of blood cells from Alzheimer disease patients.

In the penultimate session chaired by Drs. Varsha Ghandi and Arnon Lavie, analogs of purines and pyrimidines were discussed. Dr. Ghandi reported that the tumor suppressor LKB1 protects non-small cell lung cancer cells in vitro against toxicity of the purine analog 8-chloro-adenosine (8-Cl-Ado) via AMP activating protein kinase (AMPK) induction of autophagy. Dr. Lavie described the elegant design of increasingly effective dCK inhibitors based upon an iterative crystal structure-guided approach; such potent dCK inhibitors have exciting potential in treating leukemias. Dr. Antonio Vidal reported that in cultured cells, the demethylating pyrimidine analog decitabine (5-aza-2'-deoxycytidine, ada-dCyt) induces overexpression of deoxyuridine triphosphate nucleotidohydrolase (dUTPase), which may modulate cellular response to decitabine; this may be clinically relevant to decitabine treatment of myelodysplastic syndromes and acute myeloid leukemia. Dzjemma Sarkisjan presented promising in vitro cytotoxic effects and radiosensitizing effects of the novel orally available cytidine analog fluorocyclopentenylcytosine (RX-3117) in non-small cell lung cancer (NSCLC) lines by triggering apoptosis and increasing cell cycle checkpoint kinases 1 (Chk2) and 1 (Chk1). RX-3117 also downregulates DNA methyltransferase 1. Dr. Peters on behalf of Dr. Elisa Giovannetti reported intriguing evidence that the lactate dehydrogenase inhibitor (LDH) A inhibitor, NHI-1 has synergistic interactions with gemcitabine in cultured malignant pleural mesothelioma cells possibly by upregulation of hENT1.

The final session, focused on non-mammalian pyrimidine and purine metabolism, was chaired by Drs. Wolfgang Knecht and Birgitte Munch-Petersen and was fittingly dedicated to the memory of Professor Jure Piskur[8]. Dr. Munch-Petersen described Dr. Piskur's active productive investigations of purine and pyrimidine metabolism in yeast, Drosophila, and bacteria as well as his social activities. Dr. Louise Slot Christiansen reported the use of two variants of tomato thymidine kinase 1 that appear promising for application in suicide gene therapies in animal models of malignant glioma. Dr. Maria Valente described studies of *Trypanosoma brucei*, which demonstrate the importance of: dUTPase as a housekeeping enzyme, in thymidine salvage for maintaining low dUTP/dTTP ratios, and cytidine deaminase in the interconversion of pyrimidine nucleosides. Dr. Andrea López Moreno reported the cloning and characterization of kinetics of two enzymes, dihydropyrimidine dehydrogenase (DHPD) and β-ureidopropionase (β-UP), in the pyrimidine catabolic pathway of the rice *Oryza sativa*. Dr. López Moreno also described the dual roles of *Toxoplasma gondii* cytidine triphosphate synthetase (CTPase): conversion of UTP to CTP – the final step in the synthesis of cytidine nucleotides, and the first step in the formation of phospholipids.

Conclusions

PP15 was an exciting international event in which a spectrum of young and senior international investigators with diverse research expertise in purine and pyrimidine metabolism gathered to discuss exciting new information about: basic biology of non-mammalian enzymes, regulation of enzymes, transporters, receptors, therapeutic analogs, cancer chemotherapies, novel diseases such as mitochondrial disorders, as well as new therapeutic and pathomechanistic understanding of well-known conditions such as gout, hyperuricemia, inflammation, and Lesch-Nyhan syndrome. While this article summarizes the oral presentations, many important aspects of the conference not discussed here such as poster presentations, networking, and informal scientific dialogues contributed to the success of this meeting. The next meeting in Poland in 2017 will provide an outstanding venue for even more stimulating presentations in this productive field.

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Table 1

Scientific sessions of the 16th international symposium on Purine and Pyrimidine Metabolism

Session	Title	Chairs
Presymposium	Purine and Pyrimidine Translational Medicine	
1	Advances in gout and hyperuricemia	M.A. Becker (USA) and T. Takada (Japan)
2	Inborn Errors of Purine and Pyrimidine Metabolism	J.G. Puig (Spain) and M. Loeffler (Germany)
3	Purines, Pyrimidines, and Cancer	G.J. Peters (Netherlands) and L.-P. Jordheim (France)
4	Nucleoside transporters and receptors	M. Pastor (Spain) and I. Coe (Canada)
5	Purine and pyrimidine enzyme regulation	S. Eriksson (Sweden) and S. Lutz (USA)
6	Purines and pyrimidines in inflammation and autoimmune disease	B. Cronstein (USA) and W. Mehal (USA)
7	Purine and Pyrimidine metabolism and mitochondrial disorders	M. Hirano (USA) and L. Wang (Sweden)
8	Purine and Pyrimidine analogs	A. Lavie (USA) and V. Gandhi (USA)
9	Non-mammalian purine and pyrimidine metabolism	W. Knecht (Germany) and B. Munch-Petersen (Norway)
	Anne Simmonds Award Lecture	M.A. Becker (USA)

Table 2

Recipients of PPS poster awards

Presenter	Title	Academic Affiliation
Maliha Zafar	#30 Substrate translocation triggers endocytic regulation of the equilibrative nucleoside transporter 1 (ENT1)	Ryerson University, Toronto, Canada
Marie Zikanova	#42 Diagnosis of adenylosuccinate lyase deficiency by measuring of succinylpurines in neonatal dried blood spots	Charles University in Prague, Czech Republic
Makoto Hosoyamada	Urat1-Uox double knockout mice are experimental animal model of renal hypouricemia and exercise-induced acute kidney injury	Teikyo University, Tokyo, Japan