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中 文 摘 要 ： A unique feature of rheumatoid arthritis (RA) is the presence of anti-citrullinated protein antibodies (ACPA). Several risk factors for RA are known to increase the expression or activity of peptidyl arginine deiminases (PADs), which catalyze citrullination, and when dysregulated can result in hypercitrullination. However, the consequence of hypercitrullination is unknown and the function of each PAD has yet to be defined. Th cells of RA patients are hypoglycolytic and hyperproliferative due to impaired expression of PFKFB3 and ATM, respectively. Here we report that these features are also observed in PBMC from healthy at-risk individuals (ARIs). PBMC of ARIs are also hypercitrullinated and produce more IL-2 and Th17 cytokines but less Th2 cytokines. These abnormal features are due to impaired induction of PTPN22, a phosphatase that also suppresses citrullination independently of its phosphatase activity. Attenuated phosphatase activity of PTPN22 results in aberrant expression of IL-2, ATM, and PFKFB3, whereas diminished non-phosphatase activity of PTPN22 leads to hypercitrullination mediated by PADs. PAD2- or PAD4-mediated hypercitrullination reduces the expression of Th2 cytokines. By contrast, only PAD2-mediated hypercitrullination can increase the expression of Th17 cytokines. Taken together, our data depict a molecular signature of preclinical RA that is triggered by impaired induction of PTPN22.

中 文 關 鍵 詞 ： PAD2, hypercitrullination, Th17 and cytokines

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PTPN22 leads to hypercitrullination mediated by PADs. PAD2- or PAD4-mediated hypercitrullination reduces the expression of Th2 cytokines. By contrast, only PAD2-mediated hypercitrullination can increase the expression of Th17 cytokines. Taken together, our data depict a molecular signature of preclinical RA that is triggered by impaired induction of PTPN22.

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Introduction

The presence of anti-citrullinated protein antibodies (ACPA) is a unique feature of rheumatoid arthritis (RA) (1, 2). Citrullinated proteins are generated by deimination catalyzed by peptidyl arginine deiminases (PADs), including PAD1-4 and PAD6 (3). Although the etiology of RA is still not fully understood, alterations in protein citrullination probably contribute to certain risk factors in RA. For example, smoking, a major risk factor of RA, can increase the level of extracellular PAD2 and intracellular citrullinated proteins in lung lavage (4, 5). *Porphyromonas gingivalis*, the most common pathogen of periodontitis, express a PAD-like enzyme that is capable of citrullinating host proteins (6, 7). Several epidemiological studies have suggested an association between periodontitis and risk of developing ACPA or RA (8-10). In addition, a functional haplotype stabilizing the mRNA from *padi4*, the gene encoding PAD4, is associated with a higher risk of RA and the presence of ACPA in RA patients (11-14). Hematopoietic cells express mainly PAD2 and PAD4. These two PADs have overlapping but not identical substrate spectrum (15, 16). However, it is still unknown if either PAD has unique contribution to the pathogenesis of RA.

It was recently demonstrated that Th cells of ACPA+ RA patients expressed less PFKFB3, a rate-limiting enzyme of glycolysis, and ATM, a cell cycle checkpoint kinase, but a higher level of G6PD, which catalyzes the pentose phosphate pathway and promotes the generation of NADPH and glutathione (17, 18). Therefore, RA Th cells are hypoglycolytic, hyperproliferative, and under reductive stress, but the cause of these features is still unknown. In addition, it is unclear whether these features appear before or after the development of ACPA or prior to the onset of clinical symptoms. In this regard, it is notable that ACPA are found in the serum of asymptomatic individuals for an average of 3-5 years prior to the onset of clinically apparent arthritis and the classification of subjects as having RA (19, 20).

Citrullination converts positively charged arginines to neutral citrullines and is expected to alter protein folding and function. Indeed, citrullination critically regulates embryogenesis (21, 22), epithelial-to-mesenchymal transformation (23), and pluripotency of embryonic

stem cells (24). In addition, PAD4-mediated citrullination of histones is essential for the formation of neutrophil extracellular traps (NETs) (25-27), which can induce the expression of inflammatory cytokines, such as IL-6 and IL-18, as well as CCL20 and ICAM-1 from fibroblast like synoviocytes (28). NETs are also a rich source of citrullinated antigens. Thus PADs can contribute to RA pathogenesis by promoting the generation of citrullinated antigens and aggravating inflammation through the formation of NETs. However, it is still unclear if citrullination and PADs have other regulatory roles in immune cells in addition to promoting NETosis.

We recently discovered that PTPN22, a non-receptor protein tyrosine phosphatase, can interact with and inhibit the activity of PAD4 (29). This function of PTPN22 is independent of its phosphatase activity. A C-to-T single nucleotide polymorphism (SNP), located at 1858 position of human PTPN22 cDNA and replacing an arginine (R620) with a tryptophan (W620), carries the highest risk among all non-HLA genetic variations that are associated with RA (30-33). This R-to-W conversion renders PTPN22 unable to interact with PAD4 and suppress citrullination. Accordingly, the C1858T SNP is associated with hypercitrullination in peripheral blood mononuclear cells (PBMC) and heightened propensity for forming NETs even in healthy donors. Whether PTPN22 also inhibits the activity of PAD2 remains to be determined.

The frequency of the C1858T SNP in Caucasians is approximately 10%, highest among of all ethnicities. It is unclear if hypercitrullination is also present in PBMC of other at-risk individuals (ARIs) who do not carry the C1858T SNP. If it is, what is the cause and functional consequence of hypercitrullination? To answer these questions, we studied PMBC obtained from ARIs, including unaffected RA first-degree relatives (FDRs) and ACPA+ individuals without RA. Positive family history and positive ACPA status each carries an odds ratio of approximately 4-5 or higher (34-36), which is higher than that of the C1858T SNP. Thus, these individuals are at high risk of developing RA. We found in two independent cohorts of ARIs that hypercitrullination in PBMC is common. Their PBMC, regardless of ACPA status, have a defect in the induction of PTPN22, display an aberrant Th cytokine profile, and exhibit a phenotype very similar but not identical to that

of RA Th cells. We establish the chronological and causal relationships of these abnormal features, and demonstrate that attenuation of both phosphatase and non-phosphatase activities of PTPN22 caused by impaired induction of this protein is likely to be at the root of these abnormal features.

Results

Hypercitrullination in PBMC of healthy ARIs

The observation that PBMC from healthy donors carrying the C1858T SNP contain a high level of citrullinated proteins prompted us to postulate that hypercitrullination is a precondition of RA. We therefore set to determine if hypercitrullination was also detected in PBMC from healthy FDRs of RA patients recruited through the Personalized Risk Estimator for Rheumatoid Arthritis Family Study conducted at Brigham and Women's Hospital (BWH) (37). The demographic characteristics of FDRs are shown in Table 1 (BWH cohort). The level of cit-H3 in PBMC was examined with anti-cit-H3 antibody in western blotting (Figure 1A). We chose cit-H3 as readout of citrullinated proteins because we have found that the commercial anti-cit-H3 is sensitive and reliable. We also included PBMC from healthy control donors carrying (CT donors) or not carrying (CC) the C1858T SNP (Figure 1A), and osteoarthritis patients (OA in Figure 1B), treated RA patients (tRA in Figure 1B), and early/untreated RA patients (eRA in Figure 1C). These donors were recruited through the PhenoGenetic Project and Partners HealthCare Biobank (38). The level of cit-H3 was quantified with densitometry and then normalized against that of total H3 (Figure 1D). We included in each western blot 1-2 samples from other blots. By doing so, we were able to compare the level of normalized cit-H3 among experiments. We also included in the analysis 3 CC and 4 CT samples that were published previously (29). Consistent with our hypothesis, we found that PBMC from nearly all FDRs had a high level of cit-H3, even higher than that of CT donors. By contrast, PBMC from OA patients had a low level of cit-H3 comparable to that of CC donors (Figure D). Ten of the FDRs were from ACPA+ RA probands and the other ten from ACPA- RA probands. There was no difference in the level of cit-H3 between these two groups (Figure 1E). Only one FDR (#4) was positive for ACPA. Therefore, the hypercitrullination is unlikely to be related to ACPA status.

To further confirm our observation, we examined PBMC from a different cohort of ARIs recruited through the Studies of the Etiology of Rheumatoid Arthritis at University of Colorado (UC cohort, Table 1). This cohort includes 11 healthy FDRs and 3 healthy ACPA+/non-FDR individuals. It also includes 14 healthy ACPA-/non-FDR donors

(controls) and 6 early/untreated RA patients (eRA). One of the FDRs is also positive for ACPA (FDR #11). Again we detected more cit-H3 in PBMC from this cohort of ARIs compared to the 14 healthy controls (Figure 2A and 2B). There was no difference in the level of cit-H3 between ACPA+ and ACPA- ARIs (Figure 2C). There was no known current smoker among ARIs in either BWH or UC cohorts. The genotype of the C1858T SNP was determined in 12 of the 14 ARIs of UC cohort; only one carried this SNP. While the genotype of the C1858T SNP was not determined in the FDRs of BWH, its frequency is approximately 20% in RA patients in North America, so there should be no more than 5 CT FDRs in BWH. All ARIs did not have any systemic inflammation at the time of blood draw. Therefore, the elevated level of cit-H3 in ARIs is unlikely to be due to smoking, the C1858T SNP, or systemic inflammation.

If hypercitrullination in PBMC is a precondition of RA, then we should expect to see hypercitrullination in early RA patients particularly before any treatment. Consistent with our hypothesis, PBMC from early RA patients also displayed hypercitrullination compared to controls (Figure 1C, 1D, 2A, & 2B). Interestingly, no hypercitrullination was detected in 10 treated RA patients (Figure 1B & 1D). These treated RA patients had a mean CDAI of 2.6. This observation strongly suggests that effective treatment reduces the level of cit-H3. In the following experiments, ARI samples from BWH and UC were used indistinguishably. Early RA was not analyzed because they were treated soon after diagnosis.

Cellular sources of cit-H3 in PBMC

The hypercitrullination seen in ARI PBMC can come from a single or multiple populations of blood cells. To identify the source of cit-H3 in PBMC, we decided to use intracellular staining to quantify the level of cit-H3 on a single cell basis. We stimulated splenocytes collected from WT mice or mice deficient in PAD4 (PAD4KO), the only PAD bearing a nuclear localization signal, with PMA and then subjected the cells to intracellular staining of cit-H3. PMA increased the staining of cit-H3 in T, B, and non-T/non-B cells (Figure S1). This increase was almost completed abrogated in the absence of PAD4. There was still trace cit-H3 staining in PAD4KO splenic T cells. This residual staining is most likely

caused by PAD2, which is also expressed in mouse T cells and can also be found in the nucleus (39). We then stained PBMC from ARIs and control donors with anti-cit-H3 or control IgG. We excluded dead cells, and used CD3 and CD20 to identify T, B, and non-T/non-B cells (Figure 3A). Despite inter-experimental variations in the level of cit-H3 staining, we reproducibly detected more cit-H3 in T cells from ARIs compared to those from control donors (Figure 3B and 3C). No difference in the level of cit-H3 between ARIs and control donors was observed in B and non-T/non-B cells, indicating that T cells are the major contributors of cit-H3 in PBMC of ARIs.

Impaired induction of PTPN22 in ARI PBMC

As T cells are the major source of cit-H3, it is possible that ARI PBMC have a higher percentage of T cells as the cause of the higher level of cit-H3. However, in the three pairs of donors analyzed in Figure 3A and five additional pairs of donors, we did not see any major difference in the distribution of T or B cells even though the percentage of non-T/non-B cells was slightly lower in ARIs (Figure 3D). We then examined the expression of PAD2 and PAD4, two dominant PADs in hematopoietic cells (40). Interestingly, anti-CD3 stimulation led to a reduction in the transcript level of PAD2 and PAD4 (Figure 3E). However, the levels of PAD2 and PAD4 in either resting or stimulated PBCM were very comparable between controls and ARIs.

An alternative explanation for the hypercitrullination seen in ARI PBMC is impaired expression of PTPN22. We found that the level of PTPN22 transcript in resting PBMC was comparable between controls and ARIs (Figure 3F). Anti-CD3 stimulation expectedly increased the level of PTPN22 transcript by almost 2 fold in control PBMC. Surprisingly, no such induction was detected in ARIs PBMC (Figure 3F). The level of PTPN22 transcript in half of the ARIs was actually reduced by anti-CD3 stimulation. There was no difference between ACPA+ and ACPA- ARIs (Figure S2). We were able to examine the level of PTPN22 protein after anti-CD3 stimulation in 9 ARIs and 8 controls (Figure 3G). Indeed, the level of PTPN22 protein was significantly lower in stimulated ARI PBMC (Figure 3H). Contrarily, the level of cit-H3 in stimulated ARI PBMC was higher than that of stimulated control PBMC (Figure 3H). There was a reverse correlation between the level of PTPN22

and cit-H3 (Figure 3I). Those with a higher level of PTPN22 tend to have a lower level of cit-H3, suggesting that the impaired induction of PTPN22 contributes to the hypercitrullination.

Abnormal phenotype of ARI PBMC

Th cytokines, such as IL-17, IL-4 and IFN-γ, play a critical role in the pathogenesis of RA and many other autoimmune diseases. To determine if ARI PBMC have any defect in the expression of Th cytokines, the PBMC were stimulated with anti-CD3 for 24 hours and the production of Th cytokines was measured with ELISA or real time PCR. We found no difference in the level of IFN-γ between ARIs and controls (Figure 4A). Interestingly, ARI PBMC produced more IL-2 and Th17 cytokines, including IL-17A and IL-17F, but almost 50% less Th2 cytokines, such as IL-4 and IL-13. There was no difference between ACPA+ and ACPA- ARIs (Figure 4B).

A recent study indicates that naive Th cells from ACPA+ RA patients express less PFKFB3 and ATM, but more G6PD in response to stimulation (18). We found that PBMC of ARIs also had a reduced level of PFKFB3 and ATM after stimulation (Figure 4C). Accordingly, PBMC of ARIs generated less lactic acid upon stimulation (Figure 4D). The levels of PFKFB3, ATM and lactate were comparable between ACPA+ and ACPA- ARIs (Figure 4E and data not shown), suggesting that these changes do not require the development of ACPA. Interestingly, the expression of G6PD was normal in ARIs (Figure 4C). This observation indicates that the aberrant expression of G6PD is not coupled to the attenuated expression of PFKFB3 and ATM, and occurs independently of ACPA.

Distinct impacts of phosphatase and non-phosphatase activities of PTPN22

When we pooled ARI and control samples together, we found that the levels of IL-2, IL-17A, and IL-17F reversely correlated with the induction of PTPN22 (Figure 5A). There appeared to have a threshold effect for IL-2 and IL-17F. When the induction of PTPN22 dropped to below 1, the level of IL-2 and IL-17F started to rise. Contrarily, there was a positive correlation between the induction of PTPN22 and the level of ATM or PFKFB3.

There was also a trend of positive correlation between Th2 cytokines and the induction of PTPN22, but this trend did not reach statistical significance.

These observations prompted us to investigate if impaired induction of PTPN22 was responsible for the phenotype of ARI PBMC. We transfected PBMC from six randomly selected ARIs with expression vector of PTPN22 (WT in Figure 5B and 5C). The transfected cells were then stimulated with anti-CD3. In agreement with our hypothesis, forced expression of PTPN22 reduced the level of cit-H3 (Figure 5B), increased the level of Th2 cytokines, PFKFB3, ATM, and lactate, but reduced the level of IL-2 and Th17 cytokines (Figure 5C).

PTPN22 is a phosphatase and is known to attenuate activation signals in lymphocytes. Thus, impaired induction of PTPN22 is expected to augment activation signals in T cells. However, the reciprocal changes in Th2 and Th17 cytokines observed in ARI PBMC cannot be explained by stronger activation signals in T cells. In addition to acting as a phosphatase, PTPN22 also has non-phosphatase activities, including suppressing citrullination and promoting TLR-induced expression of type 1 interferon (29, 41). To determine if PTPN22 shaped the phenotype of ARI PBMC through its phosphatase or non-phosphatase activity, we also expressed W620-PTPN22 or a catalytic dead PTPN22 (CD-PTPN22) in ARI PBMC. CD-PTPN22 carries two point mutations at the protein tyrosine phosphatase domain of PTPN22 and has little phosphatase activity (42). However, it is still fully capable of suppressing citrullination (29), whereas W620-PTPN22 retains the phosphatase activity but no longer has the non-phosphatase activities (29, 41). In agreement with our published data, CD-PTPN22 but not W620-PTPN22 reduced the level of cit-H3 in ARI PBMC as efficiently as WT-PTPN22 (Figure 5B). In addition, CD-PTPN22 was able to normalize the level of Th2 and Th17 cytokines but not IL-2, ATM, PFKFB3, or lactate (Figure 5C). Although the effect of CD-PTPN22 on IL-17F did not reach statistical significance in one-way ANOVA analysis, it was statistically significant when directly compared to empty vector control in Student's t test analysis (data not shown). By contrast, W620-PTPN22 was able to normalize the level of IL-2, ATM, PFKFB3, and lactate but not Th2 or Th17 cytokines (Figure 5C). These results indicate that the aberrant

expression of IL-2, ATM, and PFKFB3 is caused by attenuated phosphatase activity of PTPN22, whereas the abnormal Th2 and Th17 cytokine profile is due to attenuated non-phosphatase activity of PTPN22.

PTPN22 regulating Th2/Th17 cytokine profile by suppressing citrullination

Attenuation of non-phosphatase activities of PTPN22 is expected to lead to hypercitrullination and impaired TLR-induced expression of type 1 interferon. The observation that normalization of Th2 and Th17 cytokines correlated with a reduction in the level of cit-H3 prompted us to postulate that hypercitrullation, but not dysregulated expression of type 1 interferon, is the cause of the abnormal Th2/Th17 cytokine profile of ARI PBMC. To test this hypothesis, we randomly selected 9 ARIs and examined the Th2 and Th17 cytokine profile of their PBMC after stimulation with anti-CD3 in the absence or presence of Cl-amidine, a pan-PAD inhibitor. Expectedly, Cl-amidine reduced the level of cit-H3 (Figure 6A). It also increased the level of IL-4 and IL-13, and decreased the level of IL-17F (Figure 6B). There was also a trend toward reducing the level of IL-17A. However, the effect of Cl-amidine treatment is rather modest. This modest effect may be expected considering that hypercitrullination has already existed in ARI PBMC before stimulation.

We therefore took two additional approaches to further examine the impact of hypercitrullination on the expression Th cytokines. We have generated a line of Jurkat cells that express an exogenous PAD2 in an inducible manner (43). We found that inducing the expression of PAD2 increased the expression of Th17 cytokines but inhibited the expression of Th2 cytokines (Figure 6C). In addition to PAD2, human PBMC also express PAD4. Attenuated non-phosphatase activity of PTPN22 may lead to hypercitrullination through PAD2 and/or PAD4. To recapitulate the cytokine phenotype of ARI PBMC and to distinguish between the impacts of PAD2-mediated and PAD4-mediated hypercitrullination, we overexpressed PAD2 or PAD4 in control PBMC, which were then stimulated with anti-CD3. Exogenous PAD2 or PAD4 equally increased the level of cit-H3 (Figure 6D), and comparably suppressed the expression of IL-4 and IL-13 (Figure 6E). However, only PAD2, but not PAD4, was able to enhance the expression of Th17 cytokines.

Discussion

A molecular signature of preclinical RA has emerged from our data (Figure S3). Impaired induction of PTPN22 leads to attenuated phosphatase and non-phosphatase activities of PTPN22. Attenuated phosphatase activity of PTPN22 results in augmented expression of IL-2 but diminished expression of ATM and PFKFB3, subsequently leading to hypoglycolysis. By contrast, attenuated non-phosphatase activity of PTPN22 causes hypercitrullination, which is responsible for aberrant production of Th2 and Th17 cytokines. PAD2- or PAD4-mediated hypercitrullination suppresses the expression of Th2 cytokines, whereas only PAD2-mediated hypercitrullination is able to augment the production of Th17 cytokines. All these molecular events very likely take place before the heightened expression of G6PD and independently of ACPA.

Unfortunately, there are only 4 ACPA+ ARIs in out study, a number probably too small to show any difference between ACPA+ and ACPA- ARIs. In addition, we were unable to examine the molecular signature in purified ARI Th cells given the limited amount of blood that we were allowed to collect from each donor. It is possible that the molecular signature is influenced by non-T cells in PBMC. This possible scenario may explain the normal expression of G6PD in ARIs. Recruiting more ACPA+ ARIs and collecting more blood from each ARI will be needed to address these issues.

The observation that PBMC of ARIs contain more citrullinated proteins further strengthens the notion that hypercitrullination is a precondition of RA regardless of the status of ACPA. Our data indicate that impaired induction of PTPN22, but not smoking, systemic inflammation, abnormal expression of PADs, or the C1858T SNP, is the cause of hypercitrullination in the ARIs of this study. Then, what is the cause of the impaired induction of PTPN22? PTPN22 is expressed mainly in hematopoietic cells and its expression in T cells is induced after anti-CD3 stimulation in vitro. There is no other SNP in addition to the C1858T within the *PTPN22* gene carrying a significant risk of RA. Thus, it is unlikely that ARIs share a SNP in the *PTPN22* gene preventing its induction by anti-CD3. We propose that yet-to-be identified environmental factors play a key role in regulating the expression of PTPN22. Recently, it was reported that gut and oral

microbiome is different between RA patients and healthy controls (44, 45). Specifically, *Prevotella copri* and *Lactobacillus salivarius* were over-represented in RA patients, whereas *Haemophilus* spp. were depleted. These observations not only highlight the critical role of the environment in the pathogenesis of RA but also raise the possibility that microbiome may influence the expression of PTPN22. Thorough investigation into the molecular mechanism regulating the expression of PTPN22 will be the first step to test this hypothesis.

Our data indicate that phosphatase and non-phosphatase activities of PTPN22 are equally important in shaping the phenotype of PBMC. However, the detailed mechanism of action of PTPN22 is still poorly understood. Attenuated phosphatase activity of PTPN22 is expected to strengthen the activation signals in lymphocytes (42, 46-48). This scenario can explain the higher level of IL-2. However, the impaired expression of ATM and PFKFB3, which are also induced by anti-CD3 stimulation, in ARI PBMC, cannot be explained by stronger activation signals and suggests a novel mechanism. The phosphatase activity of PTPN22 also inhibits the signals induced by type 1 interferon (49), modulates macrophage polarization (50), and activates inflammasome by dephosphorylating NLRP3 (51). A role of NLRP3 in Th cells was recently discovered (52). It is possible that PTPN22 promotes the expression of PFKFB3 through inflammasome in T cells. This hypothesis remains to be tested. Interestingly, several SNPs located between *pfkfb3* and *prkcq* are associated with a higher risk of RA in genome-wide association studies (53, 54). Our finding that PTPN22 directly or indirectly regulates the expression of PFKFB3 further provides a molecular link between these two RA-associated genes.

Thus far, two non-phosphatase activities of PTPN22 have been identified: suppressing citrullination and promoting LPS-induced production of type 1 interferon by myeloid cells (29, 41). The observation that the abnormal Th2/Th17 cytokine profile of ARI PBMC was partly normalized by a pan-PAD inhibitor and was recapitulated by overexpression of PADs strongly suggests that failure to suppress citrullination, but not failure to produce type 1 interferon, is the cause of the abnormal cytokine profile. In addition, the Th2/Th17 cytokine profile of ARI PBMC is almost a mirror image of the ex vivo cytokine profile of

mice treated with another pan-PAD inhibitor BB-Cl-amidine (55). While it is still unclear how hypercitrullination causes the aberrant expression of Th2 and Th17 cytokines, our data have expanded the role of hypercitrullination in RA pathogenesis. Hypercitrullination not only enlarges the pool of citrullinated antigens but also actively modulates the expression of Th cytokines.

We demonstrate again that PTPN22 is an inhibitor of citrullination. The C1858T SNP ablates this function of PTPN22, resulting in hypercitrullination and excessive production of citrullinated antigens. This mechanism can explain the synergy between the C1858T SNP and HLA shared epitopes in ACPA+ RA (56). Through hypercitrullination, this SNP also increases the propensity of forming NETs, which have been shown to play a pathogenic role in SLE and other autoimmune diseases (57, 58). This latter mechanism satisfactorily explains the association between the C1858T SNP and a higher risk of several other autoimmune diseases (59, 60), which do not have ACPA. It will be very interesting to examine the phenotype of PBMC from healthy donors carrying this SNP. As the conversion of R620 to W620 alters mainly non-phosphatase activities of PTPN22, one would expect that those PBMC should have abnormal expression of Th2 and Th17 cytokines but normal levels of IL-2, ATM, and PFKFB3.

Impaired induction of PTPN22 leads to augmented expression of Th17 cytokines and only PAD2-mediated, but not PAD4-mediated, hypercitrullination recapitulates this feature. These data strongly suggest that PTPN22 also inhibits the activity of PAD2. This scenario remains to be confirmed. The latter observation also for the very first time demonstrates that PAD2 and PAD4 have different roles in regulating the differentiation and function of Th cells. It is also consistent with previous reports showing that these two PAD enzymes have overlapping but not identical substrate spectrum (15, 16). Accordingly, PAD2-deficiency should preferentially attenuate Th17 response. However, PAD2-deficient mice are still sensitive to experimental autoimmune encephalomyelitis (61), a model of multiple sclerosis that is heavily dependent on Th17 cells. This discrepancy may originate from the fundamental difference between gain-of-function and loss-of-function approaches or from

the intrinsic difference between mouse and human. Identifying the substrates of PAD2 and PAD4 in mouse and human Th cells will clarify this issue.

Approximately 50% of the ARIs in our study had impaired induction of PTPN22. This finding is reminiscent of our recent observation showing that nearly 40% of ACPA- FDRs already had detectable levels of APCA in their sputum (62). It will be of great interest to examine if the presence of APCA in sputum correlates with the impaired induction of PTPN22 in PBMC of ARIs. While the Th cytokine profile of ARIs is different from that of controls, the difference is overall modest. This is not surprising given that all ARIs are free of systemic inflammation. However, the functional consequence of this modest difference is still unclear. The odds ratio for developing RA in our ARIs is 4-5. Accordingly, only approximately 5%-10% of the ARIs are expected to develop RA. This discrepancy strongly suggests that the molecular signature we discovered in this study is necessary but not sufficient for the development of RA and that additional "hits" are needed. One potential candidate is heightened expression of G6PD. It is a feature of Th cells from ACPA+ RA patients and positively correlated with disease activity. However, the level of G6PD was normal in the ARIs of our study, suggesting that the heightened expression of G6PD is a late event and appears after the development of the molecular signature of preclinical RA. It remains to be determined if this feature occurs before or after the onset of clinical symptoms. Longitudinally studies following ARIs will be needed to test this hypothesis.

Materials and Methods

Human subjects

Peripheral blood mononuclear cells were obtained from the following sources:

1. Brigham and Women's Hospital PhenoGenetic Project (38)
2. Partners HealthCare Biobank: an enterprise biobank of consented patients samples at Partners HealthCare (Massachusetts General Hospital and Brigham and Women's Hospital), according to IRB-approved protocols.
3. Personalized Risk Estimator for Rheumatoid Arthritis (PRE-RA) Family Study: an NIH-funded prospective, randomized controlled trial designed to evaluate whether personalized RA risk education affects willingness to change RA-related behaviors among unaffected first-degree relatives (FDRs) of RA patients (37).
4. Profiling of Cell Subsets in Human Diseases (PROSET-HD): a research initiative of Brigham and Women's Hospital comparing immune cells in the blood from patients with or without inflammatory diseases.
5. Studies of the Etiology of Rheumatoid Arthritis (SERA): a multi-center study designed to examine the role of environmental and genetic factors in the development and progression of RA-related autoimmunity (63).

ACPA status was determined with anti-CCP2 and anti-CCP3.1 ELISA assay (Diastat, Axis-Shield Diagnostics, Ltd., Dundee, Scotland, UK).

Purification, stimulation, and transfection of PBMC

Peripheral blood mononuclear cells were isolated from whole human peripheral blood by Ficoll-Paque PLUS (17-1440-03, GE Healthcare,Pittsburgh, PA) density gradient centrifugation. Transfection was performed with Amaxa nucleofection (Amaxa Biosystems, Gaithersburg, MD) according to the manufacturer's instruction. Briefly, 2-2.5 million PBMC were suspended in 100 μl of Human T cell Nucleofector solution (VPA-1002) and transfected with 5 μg of plasmid DNA. PBMC were plated at 24-well plates (2-2.5 millions/1ml/well) pre-coated with anti-CD3 (2.5 μg/ml, HIT3a clone, Biolegend, San Diego, CA) for 24 hours before harvesting.

Plasmid

The expression vectors of WT and W620 PTPN22 were described previously (29). CD-PTPN22 (D195A/C227S) was generated through site-specific mutagenesis. The expression vectors for PAD2 and PAD4 were kindly provided by Dr. Hyejeong Lee at Vanderbilt University and Dr. Anthony Rosen at The Johns Hopkins School of Medicine, (64, 65) respectively.

Intracellular cit-H3 staining

PBMC were washed twice with 1% BSA in PBS, resuspended in 100 μl of 1% BSA in PBS, and incubated with anti-CD3 and anti-CD20 at 4°C for 30 minutes. The cells were washed twice with cold PBS, fixed with 100 μl of fixation buffer (eBioscience IC Fixation Buffer) at room temperature for 20 minutes in the dark, mixed with 2 ml of permeabilization buffer (0.5% Trion X-100 in 1% BSA/PBS), centrifuged at 300-500 g and washed again with 2 ml permeabilization buffer. The washed cells were resuspended in 100 μl of permeabilizaiton buffer containing 1:300 rabbit IgG or anti-cit-H3 (ab5103, Abcam, Cambridge, MA) at 4°C in the dark for 30-60 minutes, washed twice with 2 ml permeabilizaiton buffer, and resuspended in 100 μl of permeabilization buffer containing 1:400 goat anti-rabbit IgG-PE (sc-3739, Santa Cruz Biotechnology, Dallas, TX) at 4°C in the dark for 30-60 minutes. The stained cells were washed twice with 2 ml permeabilization buffer at 4°C and resuspended in PBS for flow cytometry.

Western blotting

Whole cell extract was obtained by lysing cells with lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% TritonX-100, 0.5% DOC, 0.1% SDS, and 1 mM EDTA) containing 0.5 mM PMSF and complete protease inhibitor cocktail (Roche, Indianapolis, IN). The following antibodies were used: human PTPN22 antibody (AF3428, R&D Systems, Minneapolis, MN); citrullinated histone H3 antibody (ab5103, Abcam); histone H3 antibody (601902, Biolegend); PAD4 antibody (ab50247, Abcam); and PAD2 antibody (ab50257, Abcam). Densitometry readings of western blots were obtained and analyzed with UN-SCAN-IT 6.0 software (Silk Scientific, Inc., Orem, UT) and normalized against those of loading controls (total histone H3).

Quantitative RNA analysis

RNA isolation, reverse transcription, and real time PCR were performed as described (66). Transcript level thus detected was normalized against that of actin from the same sample. The sequences of primers used in quantitative PCR are listed in Table S1 and S2.

ELISA

Sandwich ELISA was performed with the following antibodies: anti-human IL-2 (555051)/biotin-anti-human IL-2 (555040) and anti-human IFN-γ (551221)/biotin-anti-human IFN-γ (554550) from BD Pharmingen (San Diego, CA); anti-human IL-4 (14-7049)/biotin-anti-human IL-4 (13-7048), anti-human IL-13 (14-7139)/biotin-anti-human IL-13 (13-7138), and anti-human IL-17A (14-7178)/biotin-anti-human IL-17A (13-7179) from eBioscience (San Diego, CA).

Measurement of lactate concentration

Lactate concentration was measured with the Lactate Assay Kit (K607-100, BioVision, Milpitas, CA) according to the manufacturer's instruction. Briefly, 50 μl of supernatant was mixed with 50 μl of the Reaction Mix for 30 minutes and O.D. 570 nm was measured for colorimetric assay.

Statistical analyses

Statistical analyses were performed with one-way ANOVA followed by multiple comparison tests (Figure 1D, 2B, 3E, the left panel of 3F, 5B, 5C, 6D and 6E), Student's two-tailed t test (Figure 1E, 2C, 3C, 3D, the right panel of 3F, 3H, 4, 6A, and 6B), and Spearman's correlation test (Figure 3I and 5A), * stands for $p<0.05$; ** for $p<0.01$; *** for $p<0.001$; **** for $p<0.0001$; ns for not significant. A p value less than 0.05 is considered significant. The bars shown in Figure 1D, 1E, 2B, 2C, 3H, and 4 are mean ± SD.

Study approval

This study has been approved by Partners Human Research Committee (PHRC), Boston, MA, and Colorado Multiple Institution Review Board (COMIRB), University of Colorado Denver, Anschutz Medical Campus, Aurora, CO. Informed consent was obtained from

participants prior to inclusion to the studies.

Author Contributions

HHC, GYL, HCH, and ICH designed the experiments. HHC, ND, BS, GYL, HCH conducted the experiments. PT generated Cl-amidine. HHC, ND, BS, GYL, HCH, and ICH analyzed the data. YO, JDK, KDD, MKD, JMN, JAS, DAR, EWK, and VHM recruited donors and isolated PBMC. HHC, GYL, PT, VMH, and ICH wrote the manuscript.

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References

1. Demoruelle, M.K., and Deane, K. 2011. Antibodies to citrullinated protein antigens (ACPAs): clinical and pathophysiologic significance. *Curr Rheumatol Rep* 13:421-430.
2. Uysal, H., Nandakumar, K.S., Kessel, C., Haag, S., Carlsen, S., Burkhardt, H., and Holmdahl, R. 2010. Antibodies to citrullinated proteins: molecular interactions and arthritogenicity. *Immunol Rev* 233:9-33.
3. Bicker, K.L., and Thompson, P.R. 2013. The protein arginine deiminases: Structure, function, inhibition, and disease. *Biopolymers* 99:155-163.
4. Makrygiannakis, D., Hermansson, M., Ulfgren, A.K., Nicholas, A.P., Zendman, A.J., Eklund, A., Grunewald, J., Skold, C.M., Klareskog, L., and Catrina, A.I. 2008. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. *Ann Rheum Dis* 67:1488-1492.
5. Damgaard, D., Friberg Bruun Nielsen, M., Quisgaard Gaunsbaek, M., Palarasah, Y., Svane-Knudsen, V., and Nielsen, C.H. 2015. Smoking is associated with increased levels of extracellular peptidylarginine deiminase 2 (PAD2) in the lungs. *Clin Exp Rheumatol*.
6. McGraw, W.T., Potempa, J., Farley, D., and Travis, J. 1999. Purification, characterization, and sequence analysis of a potential virulence factor from Porphyromonas gingivalis, peptidylarginine deiminase. *Infect Immun* 67:3248-3256.
7. Rosenstein, E.D., Greenwald, R.A., Kushner, L.J., and Weissmann, G. 2004. Hypothesis: the humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis. *Inflammation* 28:311-318.
8. Mikuls, T.R., Thiele, G.M., Deane, K.D., Payne, J.B., O'Dell, J.R., Yu, F., Sayles, H., Weisman, M.H., Gregersen, P.K., Buckner, J.H., et al. 2012. Porphyromonas gingivalis and disease-related autoantibodies in individuals at increased risk of rheumatoid arthritis. *Arthritis Rheum* 64:3522-3530.
9. de Smit, M., Westra, J., Vissink, A., Doornbos-van der Meer, B., Brouwer, E., and Jan van Winkelhoff, A. 2012. Periodontitis in established rheumatoid arthritis

patients: a cross-sectional clinical, microbiological and serological study. *Arthritis Res Ther* 14:R222.

10. Rutger Persson, G. 2012. Rheumatoid arthritis and periodontitis - inflammatory and infectious connections. Review of the literature. *J Oral Microbiol* 4.
11. Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M., Nagasaki, M., Nakayama-Hamada, M., Kawaida, R., Ono, M., et al. 2003. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet* 34:395-402.
12. Kang, C.P., Lee, H.S., Ju, H., Cho, H., Kang, C., and Bae, S.C. 2006. A functional haplotype of the PADI4 gene associated with increased rheumatoid arthritis susceptibility in Koreans. *Arthritis Rheum* 54:90-96.
13. Hou, S., Gao, G.P., Zhang, X.J., Sun, L., Peng, W.J., Wang, H.F., Ge, X.J., Huang, W., and Sun, Y.H. 2012. PADI4 polymorphisms and susceptibility to rheumatoid arthritis: a meta-analysis. *Mod Rheumatol*.
14. Hoppe, B., Haupl, T., Egerer, K., Gruber, R., Kiesewetter, H., Salama, A., Burmester, G.R., and Dorner, T. 2009. Influence of peptidylarginine deiminase type 4 genotype and shared epitope on clinical characteristics and autoantibody profile of rheumatoid arthritis. *Ann Rheum Dis* 68:898-903.
15. Darrah, E., Rosen, A., Giles, J.T., and Andrade, F. 2012. Peptidylarginine deiminase 2, 3 and 4 have distinct specificities against cellular substrates: novel insights into autoantigen selection in rheumatoid arthritis. *Ann Rheum Dis* 71:92-98.
16. Hensen, S.M., and Pruijn, G.J. 2014. Methods for the detection of peptidylarginine deiminase (PAD) activity and protein citrullination. *Mol Cell Proteomics* 13:388-396.
17. Yang, Z., Fujii, H., Mohan, S.V., Goronzy, J.J., and Weyand, C.M. 2013. Phosphofructokinase deficiency impairs ATP generation, autophagy, and redox balance in rheumatoid arthritis T cells. *J Exp Med* 210:2119-2134.
18. Yang, Z., Shen, Y., Oishi, H., Matteson, E.L., Tian, L., Goronzy, J.J., and Weyand, C.M. 2016. Restoring oxidant signaling suppresses proarthritogenic T cell effector functions in rheumatoid arthritis. *Sci Transl Med* 8:331ra338.

19. Rantapaa-Dahlqvist, S., de Jong, B.A., Berglin, E., Hallmans, G., Wadell, G., Stenlund, H., Sundin, U., and van Venrooij, W.J. 2003. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 48:2741-2749.

20. Nielen, M.M., van Schaardenburg, D., Reesink, H.W., van de Stadt, R.J., van der Horst-Bruinsma, I.E., de Koning, M.H., Habibuw, M.R., Vandenbroucke, J.P., and Dijkmans, B.A. 2004. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 50:380-386.

21. Esposito, G., Vitale, A.M., Leijten, F.P., Strik, A.M., Koonen-Reemst, A.M., Yurttas, P., Robben, T.J., Coonrod, S., and Gossen, J.A. 2007. Peptidylarginine deiminase (PAD) 6 is essential for oocyte cytoskeletal sheet formation and female fertility. *Mol Cell Endocrinol* 273:25-31.

22. Yurttas, P., Vitale, A.M., Fitzhenry, R.J., Cohen-Gould, L., Wu, W., Gossen, J.A., and Coonrod, S.A. 2008. Role for PADI6 and the cytoplasmic lattices in ribosomal storage in oocytes and translational control in the early mouse embryo. *Development* 135:2627-2636.

23. Stadler, S.C., Vincent, C.T., Fedorov, V.D., Patsialou, A., Cherrington, B.D., Wakshlag, J.J., Mohanan, S., Zee, B.M., Zhang, X., Garcia, B.A., et al. 2013. Dysregulation of PAD4-mediated citrullination of nuclear GSK3beta activates TGF-beta signaling and induces epithelial-to-mesenchymal transition in breast cancer cells. *Proc Natl Acad Sci U S A* 110:11851-11856.

24. Christophorou, M.A., Castelo-Branco, G., Halley-Stott, R.P., Oliveira, C.S., Loos, R., Radzisheuskaya, A., Mowen, K.A., Bertone, P., Silva, J.C., Zernicka-Goetz, M., et al. 2014. Citrullination regulates pluripotency and histone H1 binding to chromatin. *Nature* 507:104-108.

25. Leshner, M., Wang, S., Lewis, C., Zheng, H., Chen, X.A., Santy, L., and Wang, Y. 2012. PAD4 mediated histone hypercitrullination induces heterochromatin decondensation and chromatin unfolding to form neutrophil extracellular trap-like structures. *Front Immunol* 3:307.

26. Wang, Y., Li, M., Stadler, S., Correll, S., Li, P., Wang, D., Hayama, R., Leonelli, L., Han, H., Grigoryev, S.A., et al. 2009. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *J Cell Biol* 184:205-213.

27. Li, P., Li, M., Lindberg, M.R., Kennett, M.J., Xiong, N., and Wang, Y. 2010. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. *J Exp Med* 207:1853-1862.

28. Khandpur, R., Carmona-Rivera, C., Vivekanandan-Giri, A., Gizinski, A., Yalavarthi, S., Knight, J.S., Friday, S., Li, S., Patel, R.M., Subramanian, V., et al. 2013. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Sci Transl Med* 5:178ra140.

29. Chang, H.H., Dwivedi, N., Nicholas, A.P., and Ho, I.C. 2015. The W620 Polymorphism in PTPN22 Disrupts Its Interaction With Peptidylarginine Deiminase Type 4 and Enhances Citrullination and NETosis. *Arthritis Rheumatol* 67:2323-2334.

30. Kunz, M., and Ibrahim, S.M. Non-major histocompatibility complex rheumatoid arthritis susceptibility genes. *Crit Rev Immunol* 31:99-114.

31. Begovich, A.B., Carlton, V.E., Honigberg, L.A., Schrodi, S.J., Chokkalingam, A.P., Alexander, H.C., Ardlie, K.G., Huang, Q., Smith, A.M., Spoerke, J.M., et al. 2004. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 75:330-337.

32. Orozco, G., Sanchez, E., Gonzalez-Gay, M.A., Lopez-Nevot, M.A., Torres, B., Caliz, R., Ortego-Centeno, N., Jimenez-Alonso, J., Pascual-Salcedo, D., Balsa, A., et al. 2005. Association of a functional single-nucleotide polymorphism of PTPN22, encoding lymphoid protein phosphatase, with rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Rheum* 52:219-224.

33. Lee, A.T., Li, W., Liew, A., Bombardier, C., Weisman, M., Massarotti, E.M., Kent, J., Wolfe, F., Begovich, A.B., and Gregersen, P.K. 2005. The PTPN22 R620W polymorphism associates with RF positive rheumatoid arthritis in a dose-dependent manner but not with HLA-SE status. *Genes Immun* 6:129-133.

34. Koumantaki, Y., Giziaki, E., Linos, A., Kontomerkos, A., Kaklamanis, P., Vaiopoulos, G., Mandas, J., and Kaklamani, E. 1997. Family history as a risk factor for rheumatoid arthritis: a case-control study. *J Rheumatol* 24:1522-1526.

35. Hemminki, K., Li, X., Sundquist, J., and Sundquist, K. 2009. Familial associations of rheumatoid arthritis with autoimmune diseases and related conditions. *Arthritis Rheum* 60:661-668.

36. Arkema, E.V., Goldstein, B.L., Robinson, W., Sokolove, J., Wagner, C.A., Malspeis, S., Rosner, B., Grodstein, F., Karlson, E.W., and Costenbader, K.H. 2013. Anti-citrullinated peptide autoantibodies, human leukocyte antigen shared epitope and risk of future rheumatoid arthritis: a nested case-control study. *Arthritis Res Ther* 15:R159.

37. Sparks, J.A., Iversen, M.D., Miller Kroouze, R., Mahmoud, T.G., Triedman, N.A., Kalia, S.S., Atkinson, M.L., Lu, B., Deane, K.D., Costenbader, K.H., et al. 2014. Personalized Risk Estimator for Rheumatoid Arthritis (PRE-RA) Family Study: rationale and design for a randomized controlled trial evaluating rheumatoid arthritis risk education to first-degree relatives. *Contemp Clin Trials* 39:145-157.

38. Xia, Z., Liu, Q., Berger, C.T., Keenan, B.T., Kaliszewska, A., Cheney, P.C., Srivastava, G.P., Castillo, I.W., De Jager, P.L., and Alter, G. 2012. A 17q12 allele is associated with altered NK cell subsets and function. *J Immunol* 188:3315-3322.

39. Cherrington, B.D., Morency, E., Struble, A.M., Coonrod, S.A., and Wakshlag, J.J. 2010. Potential role for peptidylarginine deiminase 2 (PAD2) in citrullination of canine mammary epithelial cell histones. *PLoS One* 5:e11768.

40. Wang, S., and Wang, Y. 2013. Peptidylarginine deiminases in citrullination, gene regulation, health and pathogenesis. *Biochim Biophys Acta* 1829:1126-1135.

41. Wang, Y., Shaked, I., Stanford, S.M., Zhou, W., Curtsinger, J.M., Mikulski, Z., Shaheen, Z.R., Cheng, G., Sawatzke, K., Campbell, A.M., et al. 2013. The Autoimmunity-Associated Gene PTPN22 Potentiates Toll-like Receptor-Driven, Type 1 Interferon-Dependent Immunity. *Immunity* 39:111-122.

42. Hill, R.J., Zozulya, S., Lu, Y.L., Ward, K., Gishizky, M., and Jallal, B. 2002. The lymphoid protein tyrosine phosphatase Lyp interacts with the adaptor molecule

Grb2 and functions as a negative regulator of T-cell activation. *Exp Hematol* 30:237-244.

43. Hsu, P.C., Liao, Y.F., Lin, C.L., Lin, W.H., Liu, G.Y., and Hung, H.C. 2014. Vimentin is involved in peptidylarginine deiminase 2-induced apoptosis of activated Jurkat cells. *Mol Cells* 37:426-434.
44. Zhang, X., Zhang, D., Jia, H., Feng, Q., Wang, D., Liang, D., Wu, X., Li, J., Tang, L., Li, Y., et al. 2015. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat Med* 21:895-905.
45. Scher, J.U., Sczesnak, A., Longman, R.S., Segata, N., Ubeda, C., Bielski, C., Rostron, T., Cerundolo, V., Pamer, E.G., Abramson, S.B., et al. 2013. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. *Elife* 2:e01202.
46. Gjorloff-Wingren, A., Saxena, M., Williams, S., Hammi, D., and Mustelin, T. 1999. Characterization of TCR-induced receptor-proximal signaling events negatively regulated by the protein tyrosine phosphatase PEP. *Eur J Immunol* 29:3845-3854.
47. Ghose, R., Shekhtman, A., Goger, M.J., Ji, H., and Cowburn, D. 2001. A novel, specific interaction involving the Csk SH3 domain and its natural ligand. *Nat Struct Biol* 8:998-1004.
48. Wu, J., Katrekar, A., Honigberg, L.A., Smith, A.M., Conn, M.T., Tang, J., Jeffery, D., Mortara, K., Sampang, J., Williams, S.R., et al. 2006. Identification of substrates of human protein-tyrosine phosphatase PTPN22. *J Biol Chem* 281:11002-11010.
49. Holmes, D.A., Suto, E., Lee, W.P., Ou, Q., Gong, Q., Smith, H.R., Caplazi, P., and Chan, A.C. 2015. Autoimmunity-associated protein tyrosine phosphatase PEP negatively regulates IFN-alpha receptor signaling. *J Exp Med* 212:1081-1093.
50. Chang, H.H., Miaw, S.C., Tseng, W., Sun, Y.W., Liu, C.C., Tsao, H.W., and Ho, I.C. 2013. PTPN22 modulates macrophage polarization and susceptibility to dextran sulfate sodium-induced colitis. *J Immunol* 191:2134-2143.
51. Spalinger, M.R., Kasper, S., Gottier, C., Lang, S., Atrott, K., Vavricka, S.R., Scharl, S., Gutte, P.M., Grutter, M.G., Beer, H.D., et al. 2016. NLRP3 tyrosine

phosphorylation is controlled by protein tyrosine phosphatase PTPN22. *J Clin Invest*.

52. Arbore, G., West, E.E., Spolski, R., Robertson, A.A., Klos, A., Rheinheimer, C., Dutow, P., Woodruff, T.M., Yu, Z.X., O'Neill, L.A., et al. 2016. T helper 1 immunity requires complement-driven NLRP3 inflammasome activity in CD4(+) T cells. *Science* 352:aad1210.
53. Coenen, M.J., Trynka, G., Heskamp, S., Franke, B., van Diemen, C.C., Smolonska, J., van Leeuwen, M., Brouwer, E., Boezen, M.H., Postma, D.S., et al. 2009. Common and different genetic background for rheumatoid arthritis and coeliac disease. *Hum Mol Genet* 18:4195-4203.
54. Raychaudhuri, S., Remmers, E.F., Lee, A.T., Hackett, R., Guiducci, C., Burtt, N.P., Gianniny, L., Korman, B.D., Padyukov, L., Kurreeman, F.A., et al. 2008. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. *Nat Genet* 40:1216-1223.
55. Kawalkowska, J., Quirke, A.M., Ghari, F., Davis, S., Subramanian, V., Thompson, P.R., Williams, R.O., Fischer, R., La Thangue, N.B., and Venables, P.J. 2016. Abrogation of collagen-induced arthritis by a peptidyl arginine deiminase inhibitor is associated with modulation of T cell-mediated immune responses. *Sci Rep* 6:26430.
56. Kallberg, H., Padyukov, L., Plenge, R.M., Ronnelid, J., Gregersen, P.K., van der Helm-van Mil, A.H., Toes, R.E., Huizinga, T.W., Klareskog, L., Alfredsson, L., et al. 2007. Gene-gene and gene-environment interactions involving HLA-DRB1, PTPN22, and smoking in two subsets of rheumatoid arthritis. *Am J Hum Genet* 80:867-875.
57. Lood, C., Blanco, L.P., Purmalek, M.M., Carmona-Rivera, C., De Ravin, S.S., Smith, C.K., Malech, H.L., Ledbetter, J.A., Elkon, K.B., and Kaplan, M.J. 2016. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nat Med* 22:146-153.
58. Grayson, P.C., and Kaplan, M.J. 2016. At the Bench: Neutrophil extracellular traps (NETs) highlight novel aspects of innate immune system involvement in autoimmune diseases. *J Leukoc Biol* 99:253-264.

59. Kyogoku, C., Langefeld, C.D., Ortmann, W.A., Lee, A., Selby, S., Carlton, V.E., Chang, M., Ramos, P., Baechler, E.C., Batliwalla, F.M., et al. 2004. Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *Am J Hum Genet* 75:504-507.

60. Criswell, L.A., Pfeiffer, K.A., Lum, R.F., Gonzales, B., Novitzke, J., Kern, M., Moser, K.L., Begovich, A.B., Carlton, V.E., Li, W., et al. 2005. Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. *Am J Hum Genet* 76:561-571.

61. Raijmakers, R., Vogelzangs, J., Raats, J., Panzenbeck, M., Corby, M., Jiang, H., Thibodeau, M., Haynes, N., van Venrooij, W.J., Pruijn, G.J., et al. 2006. Experimental autoimmune encephalomyelitis induction in peptidylarginine deiminase 2 knockout mice. *J Comp Neurol* 498:217-226.

62. Willis, V.C., Demoruelle, M.K., Derber, L.A., Chartier-Logan, C.J., Parish, M.C., Pedraza, I.F., Weisman, M.H., Norris, J.M., Holers, V.M., and Deane, K.D. 2013. Sputum autoantibodies in patients with established rheumatoid arthritis and subjects at risk of future clinically apparent disease. *Arthritis Rheum* 65:2545-2554.

63. Hughes-Austin, J.M., Deane, K.D., Derber, L.A., Kolfenbach, J.R., Zerbe, G.O., Sokolove, J., Lahey, L.J., Weisman, M.H., Buckner, J.H., Mikuls, T.R., et al. 2013. Multiple cytokines and chemokines are associated with rheumatoid arthritis-related autoimmunity in first-degree relatives without rheumatoid arthritis: Studies of the Aetiology of Rheumatoid Arthritis (SERA). *Ann Rheum Dis* 72:901-907.

64. Lee, H.J., Joo, M., Abdolrasulnia, R., Young, D.G., Choi, I., Ware, L.B., Blackwell, T.S., and Christman, B.W. 2010. Peptidylarginine deiminase 2 suppresses inhibitory {kappa}B kinase activity in lipopolysaccharide-stimulated RAW 264.7 macrophages. *J Biol Chem* 285:39655-39662.

65. Harris, M.L., Darrah, E., Lam, G.K., Bartlett, S.J., Giles, J.T., Grant, A.V., Gao, P., Scott, W.W., Jr., El-Gabalawy, H., Casciola-Rosen, L., et al. 2008. Association of autoimmunity to peptidyl arginine deiminase type 4 with genotype and disease severity in rheumatoid arthritis. *Arthritis Rheum* 58:1958-1967.

66. Chang, H.H., Tai, T.S., Lu, B., Iannaccone, C., Cernadas, M., Weinblatt, M., Shadick, N., Miaw, S.C., and Ho, I.C. 2012. PTPN22.6, a dominant negative isoform of PTPN22 and potential biomarker of rheumatoid arthritis. *PLoS One* 7:e33067.

Figure legends

Figure 1. Hypercitrullination in PBMC of healthy first-degree relatives (FDRs) of rheumatoid arthritis (RA) patients recruited at Brigham and Women's Hospital. PBMC obtained from healthy FDRs (**A**), healthy donors carrying (CT) or not carrying (CC) the C1858T SNP (**A** & **B**), patients with osteoarthritis (OA) (**B**), treated RA (tRA) patients (**B**), and early/untreated RA (eRA) patients (**C**) were directly analyzed in western blots for the level of citrullinated histone H3 (cit-H3) or total histone H3 (H3). The identity of each donor within each group was denoted with Arabic numerals. The level of cit-H3 was quantified with densitometry and normalized against that of H3. The normalized density of CT#4 in **A** was arbitrarily set as 1. The normalized density of cit-H3 from all donors is shown in **D**. The normalized cit-H3 levels of FDRs from anti-citrullinated protein antibodies (ACPA)+ or ACPA- probands are shown in **E**. Statistical analysis was performed with one-way ANOVA followed by multiple comparison tests (**D**). CC group was used as the control group. Student's two-tailed t test was used in **E**.

Figure 2. Hypercitrullination in PBMC from healthy at-risk individuals (ARIs) recruited at University of Colorado. PBMC from first-degree relatives (FDR), anti-citrullinated protein antibodies (ACPA)+/non-FDR (ACPA+), early RA (eRA), and controls were analyzed in western blots for the level of citrullinated histone H3 (cit-H3) and total histone H3 (H3) (**A**). The density of cit-H3 was measured and normalized against that of H3. The normalized density of FDR donor #8 was arbitrarily set as 1. The normalized density of cit-H3 of all donors is shown in **B**. The normalized cit-H3 density from ACPA+ and ACPA- ARIs is shown in **C**. Statistical analysis was performed with one-way ANOVA followed by multiple comparison tests (**B**), and Student's two-tailed t test (**C)**.

Figure 3. Sources and causes of citrullinated histone H3 (cit-H3) in at-risk individuals (ARIs) PBMC.

A-D. PBMC were subjected to intracellular staining with control IgG or anti-cit-H3. The gating strategy is shown in **A**. The cit-H3 staining of live T, B and non-T/non-B cells is shown in **B**. The percentages of cit-H3+ T cells from three paired experiments are shown

in **C**. The percentages of T, B, and non-T/non-B cells in PBMC from 8 paired experiments are shown in **D**. **E** & **F**. PBMC from 14 ARIs and 14 control donors (con) were left unstimulated or stimulated with anti-CD3 for 24 hours. Transcript levels of PAD2 (**E**), PAD4 (**E**), and PTPN22 (**F**) were measured with real time PCR. Normalized values from the same donors are connected with lines. The fold induction of PTPN22 is shown in the right panel of **F**. **G-I**. Anti-CD3 stimulated PBMC from 9 ARIs and 8 control donors were analyzed with western blotting for PTPN22, cit-H3, and total histone H3 (H3). The density of PTPN22 and cit-H3 was normalized against that of H3 and shown in **H**. The normalized PTPN22 values were plotted against normalized cit-H3 values and shown in **I.** Statistical analysis was performed with Student's two-tailed t test in **C, D,** the right panel of **E**, and **H,** one-way ANOVA in **E** and the left panel of **F,** and Spearman's test in **I**.

Figure 4. Abnormal phenotype of at-risk individuals (ARIs) PBMC

PBMC from controls donors and ARIs (14-17 per group) were stimulated with anti-CD3 for 24 hours. The expression of indicated cytokines was quantified with ELISA or real time PCR (**A**). The levels of cytokines of anti-citrullinated protein antibodies (ACPA)+ and ACPA- ARIs are compared in **B**. In addition, the transcript levels of PFKFB3, ATM, and G6PD measured with real time PCR are shown in **C,** and the concentration of lactate in supernatant is shown in **D.** The transcript levels of PFKFB3 and ATM of ACPA+ and ACPA- ARIs are compared in **E**. Statistical analyses were performed with Student's two-tailed t test.

Figure 5. Phosphatase and non-phosphatase activities of PTPN22

A. The levels of cytokines and genes in PBMC shown in Figure 4 were plotted against the fold induction of PTPN22 shown in the right panel of Figure 3F. Statistical analysis was performed with Spearman's correlation test. **B & C**. PBMC from at-risk individuals were transfected with plasmid vector expressing PTPN22, W620-PTPN22, CD-PTPN22, or the empty vector (-), and then stimulated with anti-CD3 for 24 hours. The levels of PTPN22, citrullinated histone H3 (cit-H3), and total histone H3 (H3) in transfected/stimulated cells were quantified with western blotting (**B**). Representative blots of six independent experiments are shown in the bottom three panels of **B**. The density of PTPN22 and cit-H3

was quantified with densitometry and normalized against that of H3. The normalized levels of PTPN22 and cit-H3 are shown in the upper two panels of **B**. The expression of indicated cytokines and genes were measured with ELISA or real time PCR (**C**). The concentration of lactate in supernatant was quantified with a colorimetric assay (**C**). The data values from the same donors were connected with lines. Statistical analysis for **B & C** was performed with one-way ANOVA followed by multiple comparison tests using the empty vector-transfected groups as controls.

Figure 6. PTPN22 regulating Th2/Th17 cytokine profile by suppressing citrullination. **A & B**. PBMC from 9 at-risk individual were stimulated with anti-CD3 in the presence or absence of Cl-amidine (Cl-am, 50 μM). The level of citrullinated histone H3 (cit-H3) and total histone H3 (H3) was analyzed with western blotting (**A**). Each bracket represents one donor. The cit-H3/H3 density ratios from all donors are shown in the right panel of **A**. The levels of indicated cytokines are shown in **B**. **C**. Jurkat cells expressing (+) or not expressing (-) a doxycycline-inducible PAD2 were treated with doxycycline (50 μM) for 12 hours. The transcript levels of indicated genes from one of three independent experiments are shown. **D & E.** Control PBMC were transfected with an empty vector (-) or a vector expressing PAD2 or PAD4 and then stimulated with anti-CD3. The levels of PAD2, PAD4, cit-H3, and H3 were measured with western blotting (**D**). Representative blots from six independent experiments are shown in the left four panels of **D**, whereas normalized density of PAD2, PAD4, and cit-H3 is shown in the right three panels of **D**. The levels of indicated cytokines in the PMBC are shown in **E**. Statistical analysis was performed with paired Student's two-tailed t test (**A** & **B**) or one-way ANOVA using empty vector-transfected groups as controls (**D** & **E**).

出席國際學術會議心得報告

計畫編號	NSC 102-2320-B-040 -018 -MY3
計畫名稱	T Helper Subset Cell Activation and ACAD Dedicated by Peptidylarginine Deiminase 2
出國人員姓名 **服務機關及職稱**	劉光耀 中山醫學大學生化微生物免疫學研究所；教授
會議時間地點	2016/07/17~21, Vancouver, BC, Canada (溫哥華，加拿大)
會議名稱	(中文) 2016 第 16 屆生物化學暨分子生物學國際會議年會 (英文) The 16th International Conference on Biochemistry and Molecular Biology, 2016 (*International Union of Biochemistry and Molecular Biology: IUBMB*)
相關內容	生物化學暨分子生物和癌症免疫相關科學研究

一、　參加會議內容

2016 第 16 屆生物化學暨分子生物學國際會議年會結合生物化學暨分子生物和癌症免疫相關科學研究，其內容：

Scientific Program

SundayJuly 17, 2016

- **13:00 - 16:30**

Workshop on Biochemistry Education and Communication

Location: 8 & 15, Meeting Level, Vancouver Convention Centre East

- **17:30 - 19:30**

Plenary Session 01: Cell Death & Aging

Location: 1,2 & 3, Meeting Level, Vancouver Convention Centre East

- **19:30 - 21:00**

Welcome Reception hosted by Pascal Spothelfer from Genome BC

Location: Ballroom ABC, Lobby Level, Vancouver Convention Centre East

MondayJuly 18, 2016

- **08:30 - 10:00**

Plenary Session 02: Cancer Causes and Progression

Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **10:00 - 10:45**

Networking Break - Exhibit & Poster Viewing

Location: Ballroom ABC, Lobby Level, Vancouver Convention Centre East

- **10:45 - 12:15**

Concurrent Session 01: Metabolic Signaling in Muscle

Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **10:45 - 12:15**

Concurrent Session 02: Post-translational Modifications

Location: 11, Meeting Level, Vancouver Convention Centre East

- **10:45 - 12:15**

Concurrent Session 03: Extracellular Matrix and Signaling

Location: 8 & 15, Meeting Level, Vancouver Convention Centre East

- **10:45 - 12:15**

Concurrent Session 04: Rapid Fire Presentations - Cellular Regulation I

Location: 12, Meeting Level, Vancouver Convention Centre East

- **12:30 - 13:30**

The Art of Science Communication: An Online Approach to Science Communication Training

Location: 8 & 15, Meeting Level, Vancouver Convention Centre East

- **12:30 - 13:30**

Poster Session, Exhibits & Workshops

Location: Ballroom ABC, Lobby Level, Vancouver Convention Centre East

- **13:45 - 15:15**

Concurrent Session 05: Neurodegenerative Disease

Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **13:45 - 15:15**

Concurrent Session 06: Epigenetic Signaling & Regulation

Location: 8 & 15, Meeting Level, Vancouver Convention Centre East

- **13:45 - 15:15**

Concurrent Session 07: Novel Therapeutics

Location: 11, Meeting Level, Vancouver Convention Centre East

- **13:45 - 15:15**

Concurrent Session 08: Rapid Fire Presentations - Cellular Regulation II

Location: 12, Meeting Level, Vancouver Convention Centre East

- **15:15 - 15:45**

Networking Break - Exhibit & Poster Viewing

Location: Ballroom ABC, Lobby Level, Vancouver Convention Centre East

- **15:45 - 17:15**

Plenary Session 03: Signaling in Cell Biology & Development

Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre

- **17:15 - 18:45**

Poster Mixer Session

Location: Ballroom ABC, Lobby Level, Vancouver Convention Centre East

TuesdayJuly 19, 2016

- **08:30 - 10:00**

  Plenary Session 04: Circadian Rhythms

  Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **10:00 - 10:45**

  Networking Break - Exhibit & Poster Viewing

  Location: Ballroom ABC, Lobby Level, Vancouver Convention Centre East

- **10:45 - 12:15**

  Concurrent Session 09: Cancer Cells and Membranes

  Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **10:45 - 12:15**

  Concurrent Session 10: Epigenetic Signaling & Regulation

  Location: 8 & 15, Meeting Level, Vancouver Convention Centre East

- **10:45 - 12:15**

  Concurrent Session 11: New Technologies

  Location: 11, Meeting Level, Vancouver Convention Centre East

- **10:45 - 12:00**

  Concurrent Session 12: Rapid Fire Presentations - Cancer Origins and Treatment

  Location: 12, Meeting Level, Vancouver Convention Centre East

- **12:30 - 13:30**

  Workshop: NSERC News and Updates

  Location: 8 & 15, Meeting Level, Vancouver Convention Centre East

- **12:30 - 13:30**

Poster Session, Exhibits & Workshops

Location: Ballroom ABC, Lobby Level, Vancouver Convention Centre East

- **13:45 - 15:45**

Concurrent Session 13: Regulation of Stem Cells

Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **13:45 - 15:15**

Concurrent Session 14: Cell Death and Cell Survival

Location: 8 & 15, Meeting Level, Vancouver Convention Centre East

- **13:45 - 15:15**

Concurrent Session 15: Regulation by Photoresponses and Ions

Location: 11, Meeting Level, Vancouver Convention Centre East

- **13:45 - 15:15**

Concurrent Session 16: Rapid Fire Presentations - Neural Systems and Disease

Location: 12, Meeting Level, Vancouver Convention Centre East

- **15:15 - 15:45**

Networking Break - Exhibit & Poster Viewing

Location: Ballroom ABC, Lobby Level, Vancouver Convention Centre East

- **15:45 - 17:15**

Plenary Session 05: Signaling and Immune Function

Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **17:15 - 18:45**

Poster Mixer Session

Location: Ballroom ABC, Lobby Level, Vancouver Convention Centre East

WednesdayJuly 20, 2016

- **08:30 - 10:00**

Plenary Session 06: Regulation of RNA & Proteins

Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **10:00 - 10:45**

Networking Break - Exhibit & Poster Viewing

Location: Ballroom ABC, Lobby Level, Vancouver Convention Centre East

- **10:45 - 12:15**

Concurrent Session 17: Cancer Cells and Kinases

Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **10:45 - 12:15**

Concurrent Session 18: Apoptosis

Location: 8 & 15, Meeting Level, Vancouver Convention Centre East

- **10:45 - 12:15**

Concurrent Session 19: Metabolic Signaling

Location: 11, Meeting Level, Vancouver Convention Centre East

- **10:45 - 12:15**

Concurrent Session 20: Rapid Fire Presentations - Cardiac and Inflammatory Disease

Location: 12, Meeting Level, Vancouver Convention Centre East

- **12:30 - 13:30**

Poster Sessions & Exhibits

Location: Ballroom ABC, Lobby Level, Vancouver Convention Centre East

- **13:45 - 19:30**

Free Afternoon for Sightseeing and Special Events

Location: Delegate Services Information Desk, Convention Level, Vancouver Convention Centre East

- **19:30 - 21:00**

Conference Dinners

Location: Delegate Services Information Desk, Convention Level, Vancouver Convention Centre East

ThursdayJuly 21, 2016

- **08:30 - 10:00**

Plenary Session 07: Cancer Signaling Pathways

Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **10:00 - 10:30**

Networking Break

Location: Foyer, Meeting Level, Vancouver Convention Centre East

- **10:30 - 12:00**

Concurrent Session 21: Parasitic and Bacterial Disease

Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **10:30 - 12:00**

Concurrent Session 22: Metabolic Signaling and Diabetes

Location: 8 & 15, Meeting Level, Vancouver Convention Centre East

- **10:30 - 12:00**

Concurrent Session 23: Membrane Transport

Location: 11, Meeting Level, Vancouver Convention Centre East

- **10:30 - 12:00**

Concurrent Session 24: Rapid Fire Presentations - Inflammatory Disease and ECM

Location: 12, Meeting Level, Vancouver Convention Centre East

- **12:15 - 13:15**

Writing in Science: A Fundamental Skill for Scientists

Location: 8 & 15, Meeting Level, Vancouver Convention Centre East

- **13:30 - 15:00**

Concurrent Session 25: Neurodegenerative Disease

Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **13:30 - 15:00**

Concurrent Session 26: Post-translational Modifications

Location: 8 & 15, Meeting Level, Vancouver Convention Centre East

- **13:30 - 15:00**

Concurrent Session 27: Membrane Proteins

Location: 11, Meeting Level, Vancouver Convention Centre East

- **13:30 - 15:00**

Concurrent Session 28: Cancer Stem Cells

Location: 12, Meeting Level, Vancouver Convention Centre East

- **15:00 - 15:30**

Networking Break

Location: Foyer, Meeting Level, Vancouver Convention Centre East

- **15:30 - 17:00**

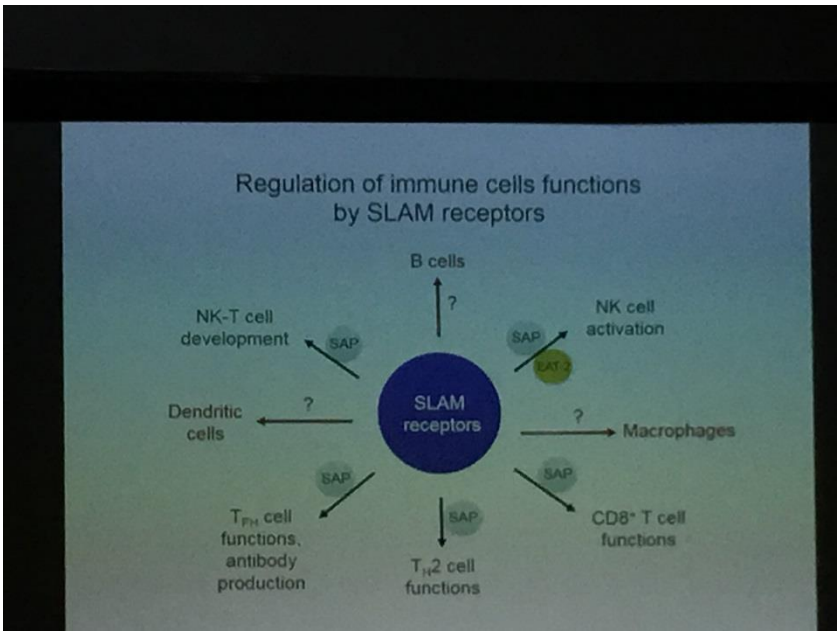
Plenary Session 08: Membrane Proteins & Channels

Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East

- **17:00 - 17:20**

Plenary Session 09: Closing Ceremony

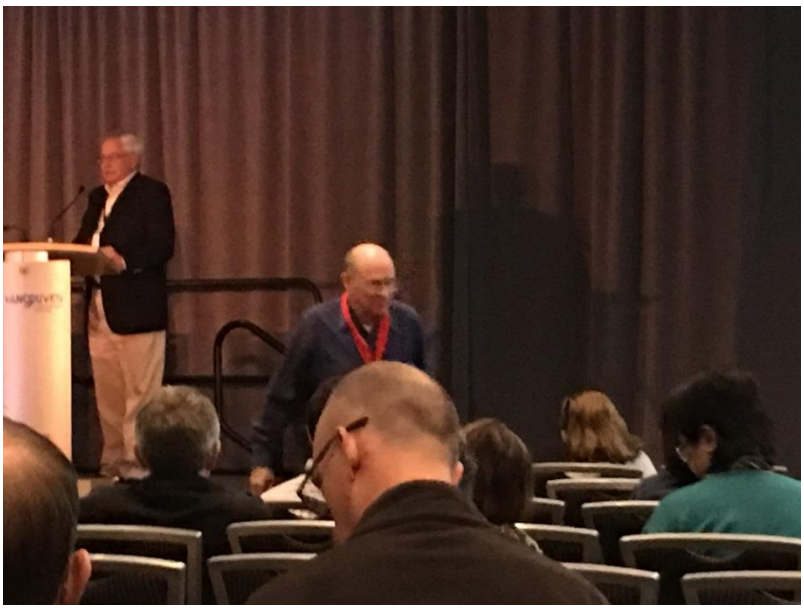
Location: 1, 2 & 3, Meeting Level, Vancouver Convention Centre East



Translational control of cancer and neurodevelopmental diseases via eIF4E

16th IUBMB conference
Vancouver

Nahum Sonenberg
McGill University
July 20, 2016



二、　與會心得

北美溫哥華大城有完整旅遊內容：自然風景、人文、原住民藝術、綠建築、空中海上飛機交通、海船運、200 年中國城歷史、市集和 UBC 大學，值得探訪。並多多鼓勵生物醫學相關科學研究人才參與國內外大型科學研究會議，交換最新穎及重要研究課題。加拿大溫哥華國際會議廳為期五天大會，北美大陸七月晴朗夏天，2016 第 16 屆生物化學暨分子生物學國際會議年會中留下了有趣主題、人物與新的靈感。細胞胞器功能互相協調、代謝、老化、癌化、疾病與免疫機制改善策略等，都在腦海中重組與堆疊，想必會有新的火花將綻放。以下註記有興趣的課題人物，以供未來研究議題交換討論與追尋。

Role of Mitochondrial Dynamics on Metabolic Homeostasis

Antonio Zorzano

Institute for Research in Biomedicine (IRB Barcelona), University of Barcelona, CIBERDEM

Biography TBA

Mechanisms and Pathologies of Protein Transport into the Endoplasmic Reticulum

Richard Zimmermann

Universität des Saarlandes, Germany

Richard Zimmermann received Dipl.Biol. and Dr.rer.nat. degrees in Biochemistry from Ludwig-Maximilians University in Munich and Georg-August University in Göttingen (both Germany), respectively. At the time he worked with Walter Neupert on the biogenesis of mitochondrial proteins, specifically the mechanism of protein import into mitochondria. In 1981 he joined William (Bill) Wickner´s laboratory at UCLA (Los Angeles, USA) as a post-doctoral fellow and focussed his attention on the biogenesis of bacterial membrane proteins. In 1983 Zimmermann became an independent research associate at Ludwig-Maximilians University in Munich and started his work on the mechanism of transport of proteins into the endoplasmic reticulum (ER) of mammalian cells. He observed that ER-import of small presecretory proteins into the ER involves cytosolic molecular chaperones and occurs independently of SRP and SRP-receptor. In 1991 he joined the medical schools of Georg-August University in Göttingen as an associate professor and in 1995 of Saarland University (Homburg, Germany) as a full professor, respectively. Since 2005 he heads the Competence Center for Molecular Medicine at Saarland University. His current work is focussed on structural and mechanistic aspects of gating of the Sec61 complex, the polypeptide conducting channel in the ER membrane, and related diseases (termed Sec61-channelopathies).

Mechanistic Diversity in Antibiotic Resistance

Gerry Wright

McMaster University, Canada

Gerry Wright is the Director of the Michael G. DeGroote Institute for Infectious Disease Research, a Professor in the Department of Biochemistry and Biomedical Sciences, an Associate member in the Department of Chemistry and the Department of Pathology and Molecular Medicine. He received his BSc in Biochemistry (1986) and his PhD in Chemistry (1990) from the University of Waterloo. He pursued his postdoctoral research at Harvard Medical School (1991-1992). In 1993, Dr. Wright joined the Department of Biochemistry at McMaster.

He holds the Michael G. DeGroote Chair in Infection and Anti-Infective Research and a Tier 1 Canada Research Chair in Antibiotic Biochemistry. From 2001-2007 Dr. Wright served as Chair of the Department of Biochemistry and Biomedical Sciences at McMaster.

Dr. Wright was elected as a Fellow of the Royal Society of Canada (2012) and a fellow of the American Academy of Microbiology (2013). He is the recipient of the Canadian Institutes of Health Research Scientist (2000-2005), Medical Research Council of Canada Scholar (1995-2000), Killam Research Fellowship (2011-1012), R.G.E. Murray Award for Career Achievement of the Canadian Society of Microbiologists, Premier's Research Excellence (1999) and the Polanyi Prize (1993). He has served on grant panel advisory boards and Chaired grant panels for a number of granting agencies in Canada, the US and Europe.

He is the author of over 230 manuscripts and is a member of the editorial boards of several scientific peer-reviewed journals including mBio, ACS Infectious Diseases, Chemistry and Biology and the Journal of Antibiotics.

Improved Mitochondrial Function in Huntington's Disease through Regulation of PDH Activity

Ana Cristina Rego

Universidade de Coimbra, Portugal

Ana Cristina Rego (ACR) received the Ph.D. in Cell Biology in 1999 at the University of Coimbra (UC), under the supervision of Prof. Catarina R. Oliveira, and was postdoctoral researcher and visiting scientist in the lab of Prof. David G. Nicholls, at the University of Dundee, Scotland, U.K., and at the Buck Institute, Novato, CA, U.S.A., from 1998-2000. ACR is tenure Assistant Professor since 1999, lecturing classes of Biochemistry, Neuroscience and Neurobiology at the Faculty of Medicine-UC where she initiated as a Teaching Assistant in 1997; in 2010 she obtained the academic degree of 'Agregação'. She is the head of "Mitochondrial Dysfunction and Signaling in Neurodegeneration" research group at the Center for Neuroscience and Cell Biology (CNC) since 2003. In 2004 and 2005 ACR coordinated the BEB PhD Programme at CNC. ACR scientific interests lie in the field of neurodegenerative diseases, by studying the cellular and molecular mechanisms of familial and age-related disorders, including Huntington's and Alzheimer's diseases. ACR is an author of 95 international peer-reviewed publications, with more than 3800 citations and an h-index of 34. She supervised and co-supervised 15 PhD students, 5 post-doctoral fellows and 25 Master students. ACR also coordinated, as Principal Investigator, 13 competitive-funding projects over the last 10 years. Funding as PI has been garnered by HighQ Foundation (USA), Lundbeck Foundation, 'Instituto de Investigação Interdisciplinar' (IIIUC), Faculty of Medicine-UC, 'Fundação para a Ciência e a Tecnologia' (FCT) projects; recently she received two awards, from 'Santa Casa da Misericórdia de Lisboa' (SCML), and 'Fundação Luso Americana para o Desenvolvimento' (FLAD). ACR is expert reviewer for 67 scientific journals. Editor of "Interaction Between Neurons and Glia in Ageing and Disease", Springer, 2007; current member of the Editorial Board of SRL Alzheimer's & Parkinson's Disease and Frontiers in Pharmacology. ACR is also the Vice-President of the board of 'Sociedade Portuguesa de Neurociências' (SPN).

Cholesterol Homeostasis in the Brain: Link to the Alzheimer's Disease

Aleksandra Mladenovic-Djordjevic

University of Belgrade, Serbia

Dr. Aleksandra Mladenovic Djordjevic received her B.Sc. and Ph.D. degrees from the University of Belgrade, Serbia. She was awarded with the Serbian government postgraduate student fellowship (1999 -2002.) among the best 200 students in the country. Her post-doctoral training was in the Laboratory for molecular neurobiology at the Institute for Biological Research "Sinisa Stankovic" (IBISS), University of Belgrade, in the field of brain ageing. She received a tenure position at the IBISS in 2003, where she is currently an Associate Professor at the Department of Neurobiology. Her research interests include synaptic plasticity in aging, molecular mechanisms of neurodegenerative diseases, cholesterol homeostasis in the brain and different therapeutic approaches in order to postpone brain ageing. Currently she leads research on molecular links between Alzheimer's disease and cholesterol metabolism supported by the Swiss National Science Foundation (collaboration with Dr. Lawrence Rajendran, University of Zurich). She is also involved as a lecturer at Neuroscience graduate program and actively mentors PhD students and is a member of dissertation Committees. She was a member of the Organizing Committee of V and VI Congress of the Serbian Neuroscience Society (2011. and 2013.) and a member of local Organizing Committee of Regional Meeting of Federation of European Neuroscience Societies (FENS2015) in Thessaloniki (Greece). Moreover Aleksandra is a representative of Serbia in several Intergovernmental frameworks for the European Cooperation in Science and Technology (COST). She is a member of the Serbian Biochemical society, Serbian Neuroscience Society and FENS. She is an Editorial Board member of Science Matters.

Mechanisms Protecting Tumor Cells from DNA Damage Induced by Chemotherapeutic Agents

Carlos Menck

Universidade de São Paulo, Brazil

Carlos Menck main research interest is on how cells respond to DNA damage, and relations with cancer and aging. He is graduated in Biology, University of São Paulo (USP), in 1977. He is Professor at the Institute of Biomedical Sciences, USP. He is Chief Editor of the Journal Genetics and Molecular Biology, and member of the Brazilian Academy of Sciences. His work investigates how DNA repair capabilities are involved in cell mutagenesis and cell death, which, in human, are directly involved in processes of carcinogenesis and aging. His projects also deal with how tumor cells respond to DNA damage after anti-tumor chemotherapy drug treatment. This may help are to potentiate the action of these drugs in cancer therapy.

Exploiting Oxidative Stress in Cancer

Tak Mak, Gairdner Award Winner (1989)

University of Toronto, Canada

Tak Wah Mak is internationally known for his work in the molecular biology of cancer and the immune system. He is a Professor in the Department of Medical Biophysics and the Department of Immunology at the University of Toronto; the Director of the Campbell Family Institute for Breast Cancer Research at the Princess Margaret Cancer Centre, and a Senior Scientist at the Ontario Cancer Institute. Dr. Mak is a world leader in the genetics of immunology and cancer. In 1984, he led the group that discovered the T cell receptor, and his published work on the cloning of T cell antigen receptor genes has been cited over 1,200 times. Since this landmark discovery, Dr. Mak has focused on elucidating the mechanisms underlying immune responses and tumorigenesis. He pioneered the use of genetically engineered mouse strains to identify genetic susceptibility factors associated with various immune disorders or different types of cancer. In particular, his team discovered that CTLA4 is a negative regulator of T cell activation (cited 2,000 times), paving the way for the development of anti-CTLA4 agents now in clinical use for autoimmune diseases. Dr. Mak's lab also made major contributions to defining the functions of PTEN (cited more than 2,000 times) as well as the relationship between the breast cancer susceptibility genes BRCA1 and BRCA2 and defects in DNA repair. Most recently, Dr. Mak's studies of the functions of various gene products in normal and cancerous cells are yielding important information on their biology that is crucial for the identification of new drug targets and the development of more effective cancer therapies. Dr. Mak was named an Officer of the Order of Canada in 2000. Other recognitions include: Gairdner Foundation International Award (Canada) Foreign Associate of the National Academy of Sciences (USA) Fellow of the Royal Society (UK) King Faisal Prize for Medicine (Saudi Arabia) Sloan Prize of the GM Cancer Foundation (USA) Paul Ehrlich and Ludwig Darmstaedter Prize (Germany).

Insulin Signals Leading to GLUT4 Translocation in Muscle Cells

Amira Klip

Hospital for Sick Children, Canada

Dr. Amira Klip received her PhD in Biochemistry at CINVESTAV in Mexico City in 1976. She did postdoctoral training at The University of Toronto, Canada (U of T) in the laboratory of Dr. David MacLennan and a second postdoctoral period at the ETH in Zurich, Switzerland. She was then hired as a research associate in Neurology at The Hospital for Sick Children in Toronto (SickKids), and in 1980 she obtained an MRC Scholarship that lounged her independent career. Dr. Klip is currently a Senior Scientist at SickKids and a Professor of Paediatrics, Biochemistry and Physiology at U of T. Dr. Klip's work deals with the regulation of glucose uptake into muscle by insulin and muscle contraction, and its failure leading to insulin resistance. In a career spanning 35 years, she has directed over 40 graduate students and 47 postdoctoral fellows, has published over 230 original papers and 80 chapters/review articles, and been a member of national and international advisory boards. She particularly enjoys her mentorship role, and was the founding director of the Research Training Centre at SickKids. She was an Associate Chief of Research at that Institution for almost 16 years. She is an active member of the Canadian research enterprise, an elected fellow of the Royal Society of Canada, the Canadian Academy of Health Sciences, and the recipient of many national and international awards. Her research operation has been supported by the Canadian Institutes of Health Research, the Banting and Best Diabetes Centre, and the Canadian Diabetes Association.

Molecular Chaperones in Protein Folding and Proteostasis Maintenance

Ulrich Hartl, Gairdner Award Winner (2004)

Max Planck Institute of Biochemistry, Germany

Ulrich Hartl received an MD and doctoral degree in Biochemistry from the University of Heidelberg. In 1985 he joined the department of Walter Neupert at Munich University where he worked on the mechanism of protein transport into mitochondria, first as a post-doctoral fellow and then as a group leader. In 1988 Hartl initiated work that resulted in the demonstration of the basic role of molecular chaperones in protein folding. The period in Neupert's laboratory was interrupted by a stay with William Wickner at UCLA, where Hartl worked on the mechanism of bacterial protein export. In 1990 he joined the faculty of Sloan-Kettering Cancer Center in New York where he investigated the mechanisms of protein folding in the bacterial and eukaryotic cytosol. He reconstituted the pathway of chaperone-assisted folding in which the Hsp70 and the GroEL chaperone systems cooperate and discovered that GroEL and its co-factor GroES provide a nano-cage for single protein molecules to fold unimpaired by aggregation. In 1993 Hartl was promoted to Full Professor with tenure, and in 1994 was made HHMI investigator. In 1997 he returned to Munich as the Director of the Department of Cellular Biochemistry at the Max Planck Institute of Biochemistry. Hartl has received several national and international awards, including the Gairdner Award in 2004, the Heineken Prize in Biochemistry and Biophysics in 2010, the Lasker Award in 2011 and the Shaw Prize in Life Science and Medicine in 2012. He is a Foreign Associate of the National Academy.

Molecular Bases of the Metabolic Programs of Neurons and Astrocytes

Juan Bolanos

Universidad de Salamanca, Spain

Juan P. Bolaños (Spain, 1964) studied and performed his PhD at the Department of Biochemistry and Molecular Biology of the University of Salamanca (Spain). After research stays in Oxford (UK) as a Biochemical Society Unilever Fellow, and in London (UK) as an EU Marie Curie post-doc (Institute of Neurology-University College London), he became Lecturer (1996) and Professor (2007) in

Biochemistry and Molecular Biology (University of Salamanca). He was the Academic Secretary of this Department, Research Vice-Dean of the Faculty of Pharmacy, President of the European Society for Neurochemistry (ESN) and, currently, council member of the Spanish Society for Biochemistry and Molecular Biology (SEBBM). He belongs to the Editorial Boards of several scientific journals, including the Biochemical Journal and the Journal of Neurochemistry. He received several awards, including the Marie Curie Excellence Award in 2005 (EU), and four Editor of the Year Awards of the Biochemical Journal. He has chaired the organizing committees of four international conferences (2007-2011), and currently chairs the SEBBM Annual Conference (Salamanca, Spain, 5-8 September, 2016). His group is interested in understanding the molecular mechanisms that regulate the energetic and redox homeostasis in the brain cells. They identified that the glycolytic-promoting enzyme PFKFB3 is subjected to ubiquitylation and proteasomal degradation by APC/C-Cdh1 (Nat Cell Biol. 2009). Currently, his group studies the molecular mechanisms responsible for the metabolic and redox adaptation of neurons and astrocytes to neurotransmission. Understanding these issues would allow identifying metabolic targets, the genetic alterations of which can contribute to neurotransmission malfunctioning and neurological problems.

mTOR Independent Regulation of Mitochondrial Metabolism and Autophagy by Akt

Dale Abel

University of Iowa, USA

Biography TBA

SLAM Family Receptors and Immune Responses

André Veillette

IRCN, Canada

Biography TBA

Nutritional Risk Factors for Colon and Breast Cancers and Multiple Sclerosis

Harald zur Hausen, Nobel Prize (2008)

German Cancer Centre, Heidelberg

Harald zur Hausen is a virologist and cancer researcher who discovered the important role that human papillomavirus plays in cervical cancer. His ground-breaking research in the 1970s and 1980s paved the way for the development of the HPV vaccine in 2006 for which he was honored with the Nobel Prize in Medicine in 2008. He also studied the Epstein-Barr virus (EBV).

Zur Hausen studied medicine at the Universities of Bonn, Hamburg, and Düsseldorf. He worked in the virus laboratories of the Children's Hospital in Philadelphia and as a senior scientist at the Institute of Wuerzburg. In 1972, he was appointed chairman and professor of virology at the University of Erlangen-Nuernberg and in 1977he moved to the University of Freiburg. From 1983 until 2003 he served as scientific director of the German Cancer Research Center. He is an elected member of various research organizations and academies.

Regulatory RNA

Andrew Fire, Nobel Prize Winner (2006)

Stanford University, USA

A native of Santa Clara County, California, Dr. Fire received training at UC Berkeley (Mathematics BA: 1975-1978), MIT (Biology Ph.D.: 1978-1983), and the Medical Research Council Laboratory in Cambridge UK (Postdoctoral: 1983-1986). From 1986 to 2003, Dr. Fire was on the staff of the Carnegie Institution of Washington's Department of Embryology in Baltimore Maryland. During his time in Baltimore, Dr. Fire assumed the position of Adjunct Professor of Biology at Johns Hopkins University. In 2003, Dr. Fire joined the faculty of the Departments of Pathology and Genetics at Stanford University School of Medicine.

eIF4E-Dependent Regulation of mRNA Translation Controls Mouse Embryonic Stem Cell Self-Renewal

Nahum Sonenberg, Gairdner Award Winner (2008)

McGill University, Canada

Nahum Sonenberg studies the molecular basis of the control of protein synthesis in eukaryotic cells and its importance in diseases such as cancer, obesity, diabetes and neurological diseases. He is known for discovering, with Aaron Shatkin, the mRNA 5' cap-binding protein, eIF4E, in 1978. He was born in Germany in 1946. He received his Ph.D. in Biochemistry from the Weizmann Institute of Science (Rehovot, Israel) in 1976, and then joined the Roche Institute of Molecular Biology in Nutley, New Jersey as a Chaim Weizmann Postdoctoral Fellow. In 1979 he moved to Montreal to become an Assistant Professor and later Professor at McGill University. Since 2002 Dr. Sonenberg is a James McGill Professor in the Department of Biochemistry and the Rosalind & Morris Goodman Cancer Research Centre, McGill University. He has received numerous awards (Wolf Prize-2014, Rosenstiel

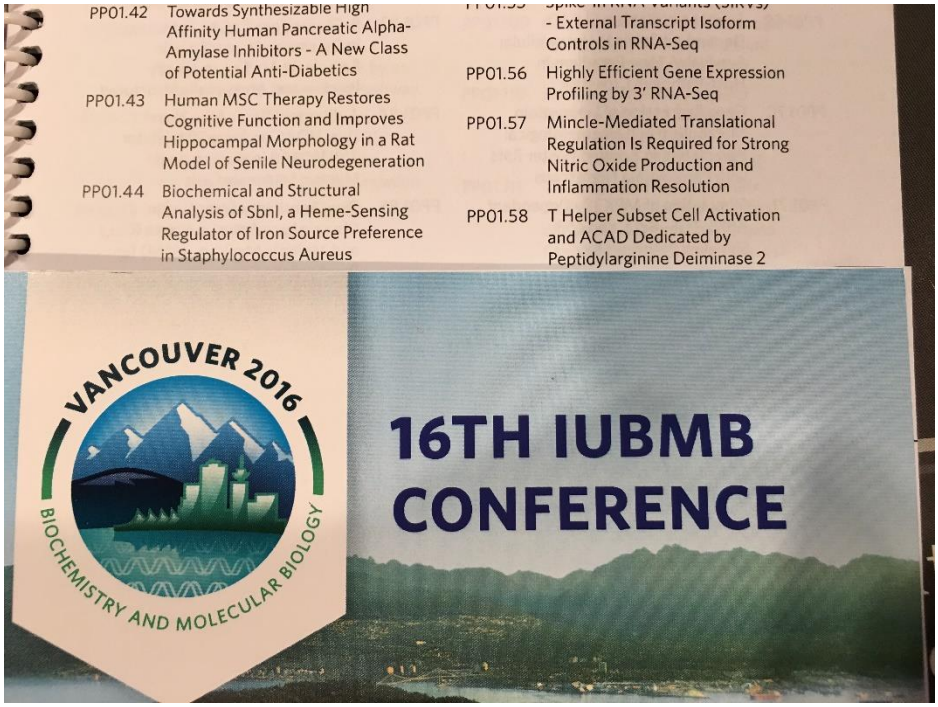
Award-2011, Gairdner Award-2008, Killam Prize-2005), and is a Fellow of the Royal Society of London (2006) and the American Association for the Advancement of Science (2012), a Foreign Honorary member of the American Academy of Arts and Sciences (2006), as well as a Foreign member of the NAS and International member of the NAM since 2015.

三、 建議

台灣具備舉辦大型研討會議城市包含六大直轄市台北、新北、桃園、台中、台南以及高雄。舉辦大型科學研討會議除了有助於城市發展與提升國家競爭力外，更提升國家經濟、科學、文化及政治交流，帶動年輕研究人員尊嚴及榮譽追尋，實質意涵可幫助年輕人傳承。

四、 攜回資料名稱及內容

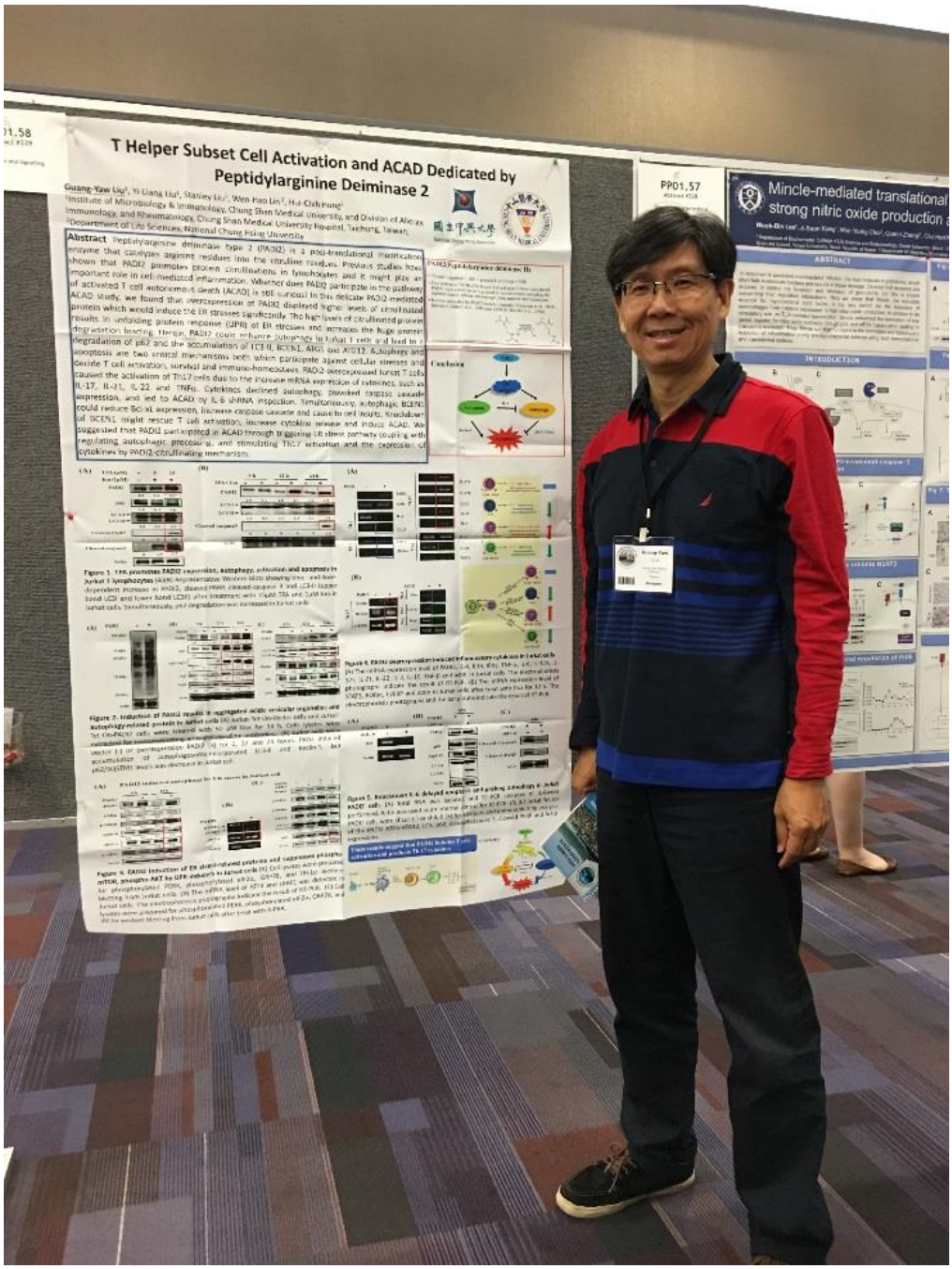
此次大議攜回討論會論文集，內容涵蓋議程及大會演講與壁報論文之題目和摘要。



五、　其他

溫哥華除了地理環境依山伴海，氣候宜人，夏季溫度攝氏 15~25 度，城市並保留大量森林公園(如 Stanley Park)，美好的風景加上清新空氣，是人與自然共存最佳寶地，台灣寶島四面環海，理應與自然共存共生，捨棄不必要的開發，留給後代子孫世世代代生存自然之地。

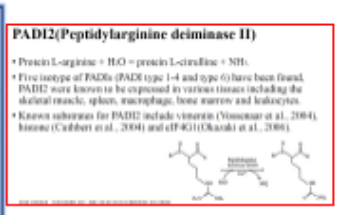
Poster

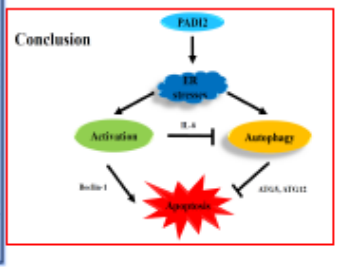

T Helper Subset Cell Activation and ACAD Dedicated by Peptidylarginine Deiminase 2
PP01.57
Mincle-mediated translational
strong nitric oxide production
ABSTRACT
INTRODUCTION

T Helper Subset Cell Activation and ACAD Dedicated by Peptidylarginine Deiminase 2

Guang-Yaw Liu[1], Yi-Liang Liu[1], Stanley Liu[1], Wen-Hao Lin [2], Hui-Chih Hung[2]
[1]Institute of Microbiology & Immunology, Chung Shan Medical University, and Division of Allergy, Immunology, and Rheumatology, Chung Shan Medical University Hospital, Taichung, Taiwan;
[2]Department of Life Sciences, National Chung Hsing University

Abstract Peptidylarginine deiminase type 2 (PADI2) is a post-translational modification enzyme that catalyzes arginine residues into the citrulline residues. Previous studies have shown that PADI2 promotes protein citrullinations in lymphocytes and it might play an important role in cell-mediated inflammation. Whether does PADI2 participate in the pathway of activated T cell autonomous death (ACAD) is still curious! In this delicate PADI2-mediated ACAD study, we found that overexpression of PADI2 displayed higher levels of citrullinated protein which would induce the ER stresses significantly. The high levels of citrullinated protein results in unfolding protein response (UPR) of ER stresses and increases the huge protein degradation loading. Herein, PADI2 could enhance autophagy in Jurkat T cells and lead to a degradation of p62 and the accumulation of LC3-II, BCEN1, ATG5 and ATG12. Autophagy and apoptosis are two critical mechanisms both which participate against cellular stresses and decide T cell activation, survival and immuno-homeostasis. PADI2-overexpressed Jurkat T cells caused the activation of Th17 cells due to the increase mRNA expression of cytokines, such as IL-17, IL-21, IL-22 and TNFα. Cytokines declined autophagy, provoked caspase cascade expression, and led to ACAD by IL-6 shRNA inspection. Simultaneously, autophagic BCEN1 could reduce Bcl-xL expression, increase caspase cascade and cause to cell insults. Knockdown of BCEN1 might rescue T cell activation, increase cytokine release and induce ACAD. We suggested that PADI2 participated in ACAD through triggering ER stress pathway coupling with regulating autophagic processing, and stimulating Th17 activation and the expression of cytokines by PADI2-citrullinating mechanism.





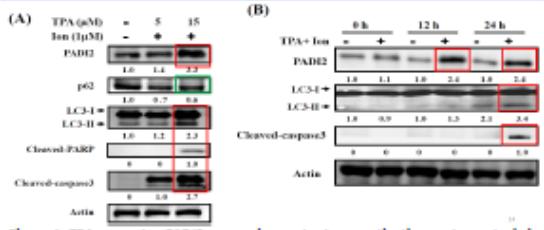


Figure 1. TPA promotes PADI2 expression, autophagy, activation and apoptosis in Jurkat T lymphocytes (A)(B) Representative Western blots showing time- and dose-dependent increase in PADI2, cleaved-PARP, cleaved-caspase 3 and LC3-II (upper band LC3I and lower band LC3II) after treatment with 15μM TPA and 1μM Ion in Jurkat cells. Simultaneously, p62 degradation was increased in Jurkat cells.

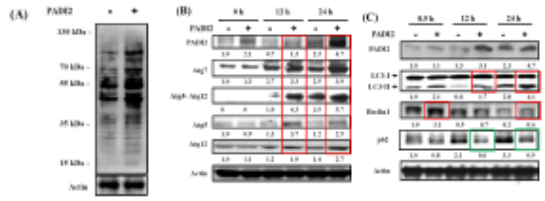


Figure 2. Induction of PADI2 results in aggregated acidic vesicular organelles and autophagy-related protein in Jurkat cells (A) Jurkat-Tet-On-Vector cells and Jurkat-Tet-On-PADI2 cells were treated with 30 μM Dox for 14 h. Cells lysates were extracted for immunoblotting with anti-citrulline antibodies. (B) Jurkat cells were vector (-) or overexpression PADI2 (+) for 0, 12 and 24 hours. PAD2 induced accumulation of autophagosome-incorporated LC3-II and Beclin-1, but p62/SQSTM1 levels was decrease in Jurkat cell.

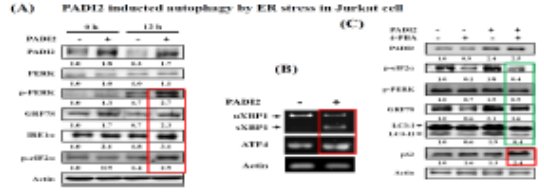


Figure 3. PADI2 induction of ER stress-related proteins and suppresses phospho-mTOR, phospho-AKT by UPR inducers in Jurkat cells (A) Cell lysates were prepared for phosphorylated PERK, phosphorylated eIF2α, GRP78, and IRE1α western blotting from Jurkat cells. (B) The mRNA level of ATF4 and sXBP1 was detected in Jurkat cells. The electrophoresis photographs indicate the result of RT-PCR. (C) Cell lysates were prepared for phosphorylated PERK, phosphorylated eIF2α, GRP78, and IRE1α western blotting from Jurkat cells after treat with 4-PBA.

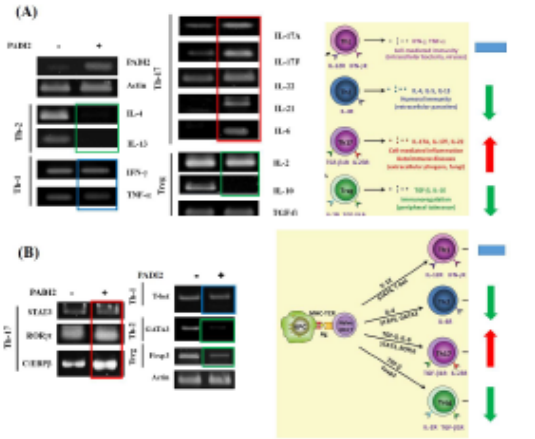


Figure 4. PADI2 overexpression induced inflammatory cytokines in Jurkat cells (A) The mRNA-expression level of PADI2, IL-4, IL13, IFNγ, TNF-α, IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-2, IL-10, TGF-β and actin in Jurkat cells. The electrophoresis photographs indicate the result of RT-PCR. (B) The mRNA-expression level of STAT3, RORγt, C/EBP and actin in Jurkat cells after treat with Dox for 12 h. The electrophoresis photographs and the bar graphs indicate the result of RT-PCR.

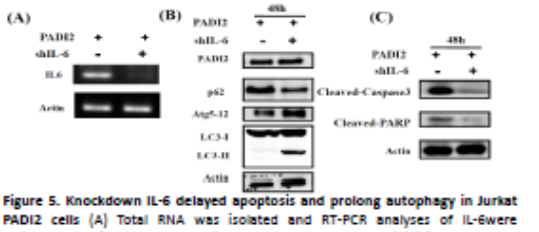
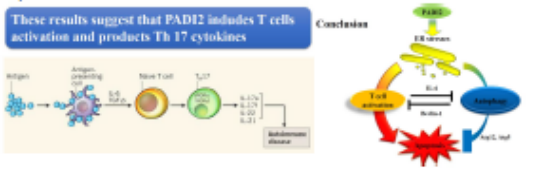
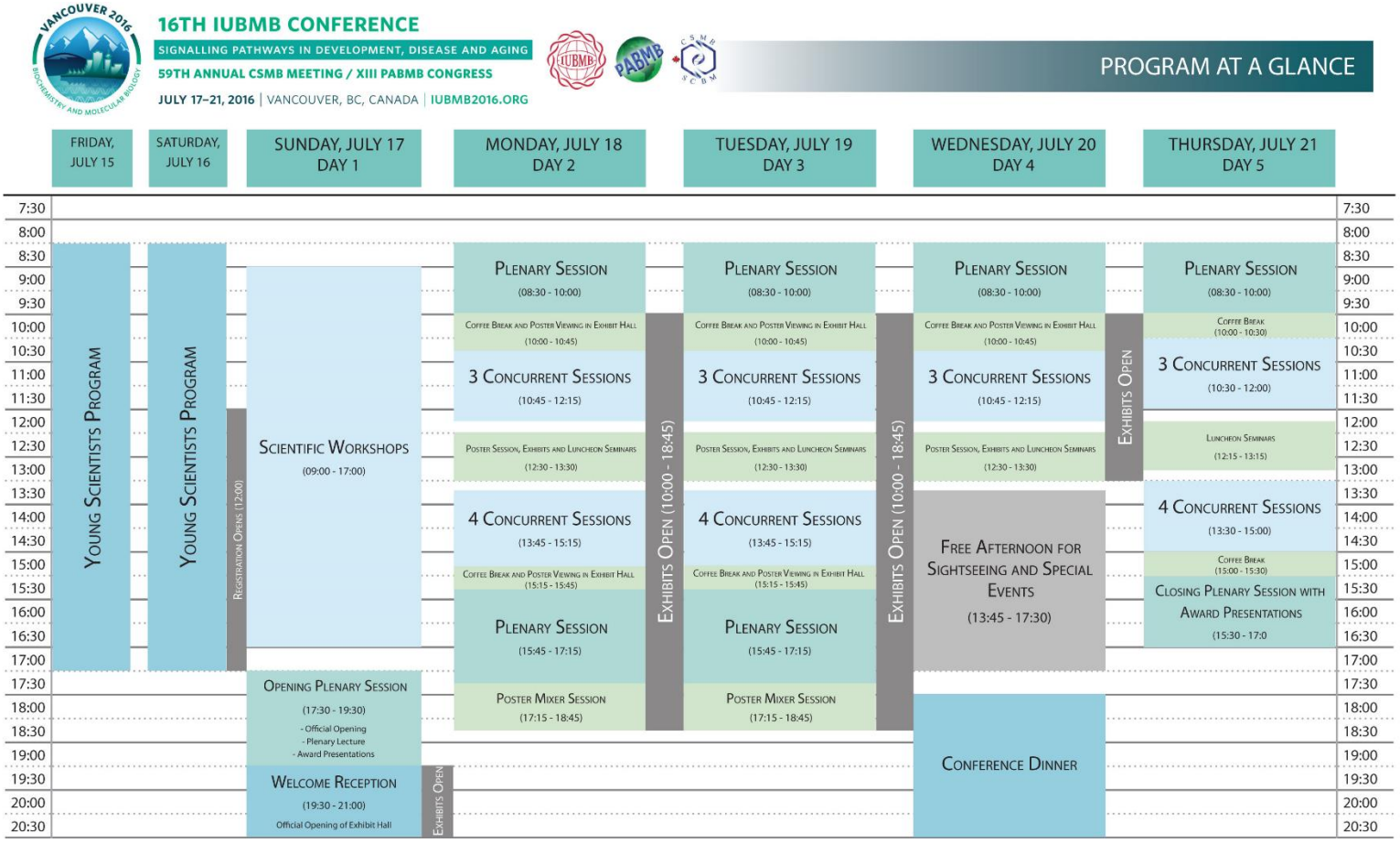


Figure 5. Knockdown IL-6 delayed apoptosis and prolong autophagy in Jurkat PADI2 cells (A) Total RNA was isolated and RT-PCR analyses of IL-6were performed. Actin was used as an internal control for RT-PCR. (B) (C) Jurkat-Tet on PADI2 cells were shLuc (-) or shIL-6 (+) for 48 hours and immunoblotting analysis of the PADI2 ATG5-ATG12, LC3, p62, cleaved-caspase 3, cleaved-PARP and Actin expression.



六、　大會議程


16TH IUBMB CONFERENCE
SIGNALLING PATHWAYS IN DEVELOPMENT, DISEASE AND AGING
59TH ANNUAL CSMB MEETING / XIII PABMB CONGRESS
JULY 17–21, 2016 | VANCOUVER, BC, CANADA | IUBMB2016.ORG
PROGRAM AT A GLANCE
FRIDAY, JULY 15
SATURDAY, JULY 16
SUNDAY, JULY 17 DAY 1
MONDAY, JULY 18 DAY 2
TUESDAY, JULY 19 DAY 3
WEDNESDAY, JULY 20 DAY 4
THURSDAY, JULY 21 DAY 5
YOUNG SCIENTISTS PROGRAM
YOUNG SCIENTISTS PROGRAM
REGISTRATION OPENS (12:00)
SCIENTIFIC WORKSHOPS (09:00 - 17:00)
OPENING PLENARY SESSION (17:30 - 19:30)
- Official Opening
- Plenary Lecture
- Award Presentations
WELCOME RECEPTION (19:30 - 21:00)
Official Opening of Exhibit Hall
EXHIBITS OPEN
PLENARY SESSION (08:30 - 10:00)
COFFEE BREAK AND POSTER VIEWING IN EXHIBIT HALL (10:00 - 10:45)
3 CONCURRENT SESSIONS (10:45 - 12:15)
POSTER SESSION, EXHIBITS AND LUNCHEON SEMINARS (12:30 - 13:30)
4 CONCURRENT SESSIONS (13:45 - 15:15)
COFFEE BREAK AND POSTER VIEWING IN EXHIBIT HALL (15:15 - 15:45)
PLENARY SESSION (15:45 - 17:15)
POSTER MIXER SESSION (17:15 - 18:45)
EXHIBITS OPEN (10:00 - 18:45)
PLENARY SESSION (08:30 - 10:00)
COFFEE BREAK AND POSTER VIEWING IN EXHIBIT HALL (10:00 - 10:45)
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PLENARY SESSION (15:45 - 17:15)
POSTER MIXER SESSION (17:15 - 18:45)
EXHIBITS OPEN (10:00 - 18:45)
PLENARY SESSION (08:30 - 10:00)
COFFEE BREAK AND POSTER VIEWING IN EXHIBIT HALL (10:00 - 10:45)
3 CONCURRENT SESSIONS (10:45 - 12:15)
POSTER SESSION, EXHIBITS AND LUNCHEON SEMINARS (12:30 - 13:30)
FREE AFTERNOON FOR SIGHTSEEING AND SPECIAL EVENTS (13:45 - 17:30)
CONFERENCE DINNER
EXHIBITS OPEN
PLENARY SESSION (08:30 - 10:00)
COFFEE BREAK (10:00 - 10:30)
3 CONCURRENT SESSIONS (10:30 - 12:00)
LUNCHEON SEMINARS (12:15 - 13:15)
4 CONCURRENT SESSIONS (13:30 - 15:00)
COFFEE BREAK (15:00 - 15:30)
CLOSING PLENARY SESSION WITH AWARD PRESENTATIONS (15:30 - 17:0
7:30 8:00 8:30 9:00 9:30 10:00 10:30 11:00 11:30 12:00 12:30 13:00 13:30 14:00 14:30 15:00 15:30 16:00 16:30 17:00 17:30 18:00 18:30 19:00 19:30 20:00 20:30

科技部補助計畫衍生研發成果推廣資料表

日期:2016/10/29

科技部補助計畫	計畫名稱: 台灣本土五葉松活化免疫與抑癌成份之評估研究
	計畫主持人: 劉光耀
	計畫編號: 102-2320-B-040-018-MY3　　學門領域: 保健營養

無研發成果推廣資料

102年度專題研究計畫成果彙整表

計畫主持人：劉光耀	**計畫編號：**102-2320-B-040-018-MY3
計畫名稱：台灣本土五葉松活化免疫與抑癌成份之評估研究	

成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			1	篇	
		研討會論文			1		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		

	技術移轉	件數	0	件	
		收入	0	千元	
參與計畫人力	本國籍	大專生	0	人次	
		碩士生	1		
		博士生	0		
		博士後研究員	1		
		專任助理	0		
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）					

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   論文：■已發表　□未發表之文稿　□撰寫中　□無
   專利：□已獲得　□申請中　■無
   技轉：□已技轉　□洽談中　■無
   其他：（以200字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）
   研究成果學術價值提供新穎疾病分子機制及可能天然物活化免疫和疾病預防應用價值

4. 主要發現
   本研究具有政策應用參考價值：■否　□是，建議提供機關
   （勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
   本研究具影響公共利益之重大發現：■否　□是
   說明：（以150字為限）