Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

p-21 activated kinase as a molecular target for chemoprevention in diabetes

Kyle Dammann PhD[#], Vineeta Khare PhD[+], Clyde Coleman PharmD*, Henrik Berdel M.D.**, Christoph Gasche M.D.[+]

[#]Medical University of the Americas; Charlestown, Nevis, West Indies. *University of Kentucky HealthCare, Clinical Surgery Staff Pharmacist; Lexington, Kentucky. **University of Kentucky Healthcare Section on Acute Care Surgery and Trauma Department of Surgery; Lexington, Kentucky. [+]Medical University of Vienna, Department of Internal Medicine III; Vienna, Austria.

Running Title: PAK1 in chemoprevention and diabetes

Correspondence: Kyle Dammann, PhD. Phone: 502-370-1867; email: dammannkw@gmail.com

Word Count: abstract 259; manuscript 3361; references 138; tables 6; supplementary tables 1; figures 5; supplementary figures 3

© 2018 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

ABSTRACT

Hypothesis: Anti-diabetic drugs modulate p-21 activated kinase (PAK) signaling

Introduction: Type 2 diabetes mellitus (T2DM) is a chronic inflammatory disease associated with increased cancer risk. PAK signaling is implicated in cellular homeostasis when regulated, and cancer when unrestrained. Recent reports provided a role for PAK signaling in glucose homeostasis, but the role of PAKs in the pathogenesis of T2DM is unknown. Here we explored whether PAK signaling should be targeted via chemoprevention to reduce diabetes-associated cancer risk.

Methods: PAK interacting partners in T2DM were identified using online STRING database. Systematic literature review provided the effect of anti-diabetic drugs on PAK signaling. Review of clinical studies revealed the overall clinical response rate and percentage of adverse events in piogliazone (n=53) and metformin (n=91) treated patients with PAK-dependent diseases.

Results: A total of 30 PAK interacting partners were identified (10: reduced beta-cell mass; 10: beta-cell dysfunction; 10: obesity-insulin resistance) which were highly associated with Wnt, and G-protein signaling. Anti-diabetic drug metformin activated signaling pathways upstream; whereas pioglitazone inhibited pathways downstream of PAK. Overall clinical response upon pioglitazone treatment was 53%. 79% of pioglitazone and 75% of metformin treated patients had adverse events. Pioglitazone reduced molecular-PAK biomarkers of proliferation (Ki67 and CyclinD1), and metformin had the opposite effect.

Conclusions: PAK signaling in T2DM involves Wnt and G-protein signaling which is altered by anti-diabetic drugs metformin and pioglitazone. Apart from

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

48 the therapeutic limitations of adverse events, pioglitazone is promising in
49 chemoprevention, however long-term multi-centered studies, which initiate
50 pioglitazone treatment early will be required to fully assess the full potential of
51 these drugs.

52 **Keywords:** p-21 activated kinase; pioglitazone, metformin, type 2 diabetes
53 mellitus, cancer, chemoprevention, and inflammation.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

INTRODUCTION

Type 2- diabetes mellitus (T2DM) is a global epidemic and its prevalence is continually increasing, especially in the western world. The etiology of T2DM is intimately linked to obesity, genetics, and sedentary lifestyle. Disease manifestations including blindness and neuropathy decrease quality of life in diabetic patients, and long-term complications such as cardiovascular disease and renal failure, ultimately result in death (McCarthy, 2010).

Although the pathogenesis of T2DM is multifactorial and complex, its current understanding encompasses hepatic insulin resistance, dysfunctional insulin signaling, abnormal glucose metabolism, and persistent hyperglycemia (McCarthy, 2010). In addition to disease specific complications, several consequences of hyperglycemia have been described such as an aberrant immune response, chronic inflammation, and tumorigenesis (Chang & Yang, 2016). Persistent diabetes increases the lifelong risk of developing pancreatic, liver, and colorectal cancer and also fuels the tumor microenvironment in cancer patients (Chang & Yang, 2016).

Anti-diabetic drugs such as the biguanides, sulfonylureas, and glitazones have provided the current basis of understanding in the clinical management of T2DM however little is known regarding whether these drugs are also effective in reducing the associated cancer risk . Effective chemoprevention in T2DM will target processes involved in both glucose metabolism and carcinogenesis.

Molecular targets with emerging roles in both cancer and diabetes include a family of six different kinases, the p-21 activated kinases (PAKs) (Dammann et al., 2014). Here we investigated PAK overexpression in T2DM, the effect of anti-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

diabetic drugs on PAK signaling, and further explored PAK as a molecular target in cancer chemoprevention in T2DM.

METHODS

Molecular analysis of PAK signaling pathways and their involvement in response to anti-diabetic drugs

A systematic literature review and molecular analysis of pre-clinical studies using online library Pubmed (https://www.ncbi.nlm.nih.gov/pubmed) was performed to establish upstream and downstream PAK targets. Key words included (PAK signaling or p21 activated kinases in addition to the following targets: AMPK, RAS, mTOR, PI3K/AKT, RAC1, CDC42, MAPK, p38, JNK, NF-kB, PPAR-gamma, ROS, VEGF, Wnt/Beta-catenin). Similar studies were screened for PAK signaling pathways involving known targets (above) of glucose homeostasis, inflammation, proliferation, survival, and angiogenesis. Both upstream and downstream PAK targets were identified in each of these pathways, and the effect of PAK signaling targets on inflammation, proliferation, survival, and angiogenesis was evaluated. Studies in the literature involving anti-diabetics drugs (glitazones, metformin, glyburide) were further analyzed for their effect on PAK signaling pathways (inflammation, proliferation, survival, angiogenesis) on targets upstream and downstream of PAKs.

Identification of PAK interacting partners

PAK interacting partners were identified using string database (www.string-db.org) as seen in (Szklarczyk et al., 2015)(Campregher et al., 2012). Confidence was set to 0.40 and active prediction methods, neighborhood, gene fusion, co-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

occurrence, co-expression, experiments, database, and text mining analysis were performed. Interacting partners were identified in three different conditions which may predispose to T2DM using targets of reduced beta-cell mass (CDKAL1, CDKN2A, CDKN2B), beta cell dysfunction (MTNR1B, TCF7L2, KCNJ11), and obesity/insulin resistance (FTO, IRS1, PPARG) as reported (McCarthy, 2010). Novel PAK interacting partners were further investigated and their pathophysiological role in T2DM was investigated and references for further exploration of targets were provided. All PAK partners were analyzed according to the molecular pathway involved in T2DM.

Clinical study inclusion/exclusion criteria and systematic review

Human clinical studies were identified on ClinicalTrial.gov. Inclusion criteria consisted of observational or interventional studies using metformin or pioglitazone in patients with diseases previously identified in the literature to have increased PAK expression levels such as bladder, leukoplakia, lung, prostate, esophageal, and colorectal cancer (Dammann et al., 2014). Studies with molecular biomarker analysis or targets downstream of PAKs were included. Studies without data were excluded from analysis. Clinical analyses of overall response rate, adverse events, as well as molecular analysis of biomarkers were performed on pooled data from pioglitazone or metformin treated patients.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

RESULTS

PAK signaling is associated with diabetes and cancer

Previous studies have provided a role for PAK in both diabetes and cancer, however a clear depiction of the signaling pathways involved in both diseases has not been performed. Systematic review of the literature demonstrated 14 PAK signaling pathways, either upstream or downstream of PAK, and 11 or 78% of these pathways were also associated with glucose homeostasis (Table 1 & 2; Supplementary Figure 1 & 2). All of the PAK signaling pathways which were associated with glucose homeostasis were also associated with malignant inflammatory, proliferative, survival, and angiogenic signaling which occurs in diseases such as cancer (Table 3 and Supplementary Figure 3).

PAK interacting partners are associated with the pathogenesis of T2DM

The pathogenesis of T2DM was previously linked to obesity, insulin resistance, and molecular alterations of pancreatic beta cells (McCarthy, 2010). PAK signaling mediates pathways involved in glucose homeostasis (Table 2); but whether PAK or its partners are actually involved in the pathogenesis of T2DM is unknown. We identified protein-protein interactions and prospective interacting partners in the pathogenesis of T2DM by screening PAKs 1-6 and previously identified markers of reduced beta cell mass (Figure 1A), dysfunction (Figure 1B), and obesity and insulin resistance (Figure 1C), using the online STRING database (Szklarczyk et al., 2015).

Thirty total PAK interacting partners were found to involve reduced Beta-cell mass (10), Beta-cell dysfunction (10), and obesity and insulin resistance (10) (Figure 1D and Supplementary Table 1). Signaling pathways associated with PAK

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

interacting partners included cell cycle control, receptor tyrosine kinases (RTK), G-proteins, and Wnt signaling (Figure 1E). Reduction in Beta-cell mass was exclusively linked to eight-interacting partners involving cell cycle control. Beta-cell dysfunction was found to be associated with four-interacting partners linked to Wnt signaling, and six-interacting partners involving RTKs were associated with obesity/ insulin resistance (Figure 1E). G-protein signaling involved two-interacting partners involved in reduced Beta-cell mass, five-partners in Beta-cell dysfunction, and four-partners in obesity/insulin resistance (Figure 1E). These data suggest PAK interacting partners are associated with and utilize the cell cycle, Wnt, RTK, and G-protein signaling in the pathogenesis of T2DM.

Upstream and downstream PAK signaling pathways are utilized by anti-diabetic drugs pioglitazone and metformin

We found that PAK interacting partners were involved in the pathogenesis of T2DM, however the role of PAK signaling in T2DM remained elusive. We investigated this by analyzing the effect of anti-diabetic drugs metformin (met), glyburide (gly), and glitazones (glit) on PAK signaling pathways (Table 4). Overall, glit and met each altered ten PAK signaling pathways whereas gly altered three (Figure 2 A). Further analysis revealed all three anti-diabetic drugs altered pathways upstream and downstream of PAK (Figure 2B). Glit exerted the most profound effect on downstream PAK signaling pathways [glit=9 vs met=7 vs gly=3]. Met altered more pathways upstream of PAK [met=5 vs glit=3 vs gly=1] (Figure 2B).

Malignant PAK signaling is involved in disease pathogenesis. To further understand the role in which met, gly, and glit interfere with PAK signaling in T2DM, we asked if any of these drugs activate or inhibit inflammatory,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

proliferative, survival, or angiogenic pathways upstream and downstream of PAK (Figure 2C-F). Differential pathway analysis revealed met and glit consistently altered more pathways than gly (Fig 2C-F). Glit inhibited more pathways downstream of PAK than met in [inflammation: glit=7 vs met=4], [proliferation: glit=8 vs met=4], [survival: glit=8 vs met=4], [angiogenesis: glit=8 vs met=4] (Figure 2C-F). However, met activated more pathways than glit which are exaggerated upstream of PAK and involved in inflammation [met=4 vs glit=2;], proliferation [met=2 vs glit=0], survival [met=3 vs glit=1], and angiogenesis [met=3 vs glit=1] (Figure 2C-F). These data suggest that anti-diabetic drugs alter PAK signaling. Metformin activates pathways upstream and glitazones inhibit pathways downstream of PAK.

Pioglitazone and metformin have therapeutic limitations in cancer patients with PAK overexpression

Previous clinical trials have attempted to establish whether met and pio therapeutically have chemopreventive activity in humans, but the results remain highly ambiguous. We showed that met and pio altered PAK signaling upstream and downstream of PAK, and asked whether the chemopreventive nature of these drugs was found in diseases with PAK overexpression. To investigate this, we analyzed clinical studies in diseases known to overexpress PAK in which met or pio treatment was tested (Table 5).

Initially, we screened hundreds of studies on ClinicalTrials.gov for diseases with PAK overexpression and either met or pio treatment. We found one observational-prospective cohort and six interventional studies (three single arm and three randomized double blind) with these criteria (Table 5 and Figure 3A).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

We investigated the effect of anti-diabetic therapy in patients with PAK over-expression by calculating an overall clinical response rate (OCRR), which was defined as the total number of patients who had ≥ 50% decrease in the sum of all their lesions post-treatment. The OCRR in pio-treated patients was 53% (Figure 3B); 28/53 patients responded and 25/53 did not. The corresponding data for met were unavailable. To further evaluate patient outcome, we calculated the number of serious or other adverse events after pio and met treatment (Figure 3B). Comparison of pio and met treatment revealed a higher percentage of serious [pio: 3/53 = 5.7% vs met: 3/91 = 3.3%] and other [pio: 42/53 = 79% vs met: 68/91 = 74.7%] adverse events upon treatment with pio (Figure 3B). Additionally, in comparison to patients treated with met, a fewer percentage of pio-treated patients were without adverse effects [pio: 11/53 = 21% vs met: 25/91 = 27.4%] (Figure 3B). Pioglitazone associated events included edema (15%), oral pain (13%), and hypertension (7.5%), while those associated with metformin were gastrointestinal symptoms like constipation (7%), diarrhea (23%), and nausea (13%). These data suggest that patients with tumors which are known to have PAK overexpression have a good clinical response to pio; and both pio and met treatment are associated with a high rate of mostly mild adverse events.

Pioglitazone and metformin alter biomarkers downstream of PAK in human disease

We found that patients which have diseases overexpressing PAK responded to pio, however it was unclear whether pio or met actually interfered with PAK signaling. We therefore analyzed biomarkers downstream of PAK signaling pathway involved in apoptosis, cell cycle, PI3K/mTOR, and PPARγ (Table 6).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

We calculated the percent change in biomarker expression in pio and met treated patients to visualize the molecular effect on PAK signaling (Figure 4). Molecular analysis revealed that pio treatment decreased expression levels of multiple biomarkers including, apoptotic marker BCL2, and cell cycle markers CyclinD1 and Ki67, respectively. Pio treatment increased total and cytoplasmic PPARγ levels by 50% and 8%, however it decreased nuclear expression by 32% (Figure 4). Met treatment resulted in an increase in cell cycle biomarker Ki67, and did not alter expression levels of PI3K/mTOR markers PS6K1 or PS65Ser235 (Figure 4). These data indicate that pio inhibited pathways involved in apoptosis and cell cycle regulation whereas met stimulated or did not alter these pathways in patients which potentially have aberrant PAK signaling.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

DISCUSSION

Type 2 diabetes is a major cause of disability and death worldwide, and in addition to its multiple disease specific complications, it also carries and increased cancer risk, such as colon cancer (Chang & Yang, 2016). P-21 activated kinases are serine-threonine kinases, which influence multiple cell functions from normal cell signaling to cancer (Kumar et al., 2016). Physiological PAK signaling is regulated and implicated in the maintenance of cellular homeostasis, however as the extracellular microenvironment or PAK expression in disease changes, physiological signaling becomes pathological (Figure 5). Here we reviewed physiological PAK signaling and found it is highly associated with glucose homeostasis, although PAKs role in the pathogenesis of T2DM, and cancer in diabetes patients is largely unknown.

PAK overexpression is associated with disease severity; however, in diabetes, Ahn et al. previously reported a reduction in PAK1 expression in beta cells of the pancreas (Ahn et al., 2016). Here we emphasized the importance of PAK signaling (kinase activity) versus total expression levels, as both chronic inflammation and hyperinsulinemia associated with T2DM (Chang & Yang, 2016) (Donath & Shoelson, 2011), likely leads to pancreatic and peripheral PAK activation. Additionally, six different PAKs have been characterized; therefore signaling from other PAKs may compensate for reduction in PAK1 expression. We identified novel PAK interacting partners associated with the pathogenesis of T2DM, involving reduced beta cell mass and dysfunction, and obesity-insulin resistance (Figure 1). Interestingly, we identified PAK interacting partner IQGAP1 was involved in all three pathways (Figure 1D). IQGAP1 acts as a

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

molecular scaffold for small Rho-GTPase activation of PAKs (R. Li et al., 1999), which further implicates the importance of PAK kinase activation in T2DM.

The initial pathogenesis of T2DM involves beta cell expansion to compensate for hyperglycemia, which eventually may lead to reduced beta cell mass (McCarthy, 2010). Here, PAK interacting partners involved with reduced beta cell mass and dysfunction were specifically associated with cell cycle regulation and Wnt signaling (Figure 1D). It is possible that in early diabetes, hyperglycemia promotes cell cycle progression via PAK- Beta-catenin signaling (Park et al., 2012). Over time, PAK driven proliferation and inflammation may lead to oxidative stress (Reuter et al., 2010) and therefore contribute to beta cell dysfunction and subsequent reduction in beta cell mass (Drews et al., 2010). Interestingly, in oxidative stress nuclear Beta-catenin was reported to associate with FOXO transcription factors (Essers et al., 2005), which could potentially modulate PAK expression levels (Mazumdar & Kumar, 2003) in line with reports from Ahn et al. Another explanation for PAK's role here may involve an interaction with tumor suppressor p53 (Figure 1A and D). Interestingly, activation of both p53 and MDM2, a p53 ubiquitin ligase, was reported in T2DM (Xiaomu et al., 2016), and a PAK–MDM2 interaction was previously described (T. Liu et al., 2013). Others provided a role for PAK upstream of p53 (Murray et al., 2010) (Park et al., 2009), thus PAK signaling in T2DM may activate p53 directly, or indirectly via MDM2 or through a cell stress pathway such as oxidative stress or stress associated MAPK like p38/JNK (Shi et al., 2014) thereby inducing apoptosis, impeding cell cycle progression, and subsequently reducing beta cell mass. Additional PAK interacting partners associated with pathways involving obesity-insulin resistance involved targets in RTK signaling, which is likely a

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

consequence of multiple growth factors, and the chronic inflammatory state associated with obesity in T2DM (Donath & Shoelson, 2011).

We sought to illuminate the role of PAK signaling in T2DM by analyzing the effect that three well-known classes of anti-diabetic drugs had on PAK signaling (Figure 2). Biguanide (metformin), sulfonylurea (glyburide), and glitazone (pioglitazone) all interfered with signaling upstream and downstream of PAKs, and this effect was more apparent with metformin (upstream) and pioglitazone (downstream) of PAKs (Figure 2). Both metformin and pioglitazone mediated inflammatory, proliferative, survival, and angiogenic pathways associated with PAKs (Table 4 and Figure 2).

Considering the role of PAK signaling in the initiation of disease (Kumar et al.,, 2016), and that PAK signaling appears to be activated in T2DM (Table 2), and inactivated by anti-diabetic drugs (Figure 2), we investigated whether metformin or pioglitazone were beneficial in patients with PAK-overexpressing diseases including oral cancer (Parvathy et al., 2016), non small cell lung cancer (Y. Liu et al., 2016), prostate (T. Liu et al., 2013), esophageal cancer (Gan et al., 2015), bladder (Ito et al., 2007), and colorectal cancer (Song, Wang, Zheng, Yang, & Xu, 2015). Pre-clinical studies investigating the chemopreventive effects of these drugs seemed promising (Girnun et al., 2002) (Grommes et al., 2013) (Burotto & Szabo, 2014), however results from human studies remain highly ambiguous (Lewis et al., 2015) and a more complete analysis of this data would allocate whether inhibition of PAK by metformin or pioglitazone is a promising for chemoprevention.

We performed a systematic review of several studies (Table 5) involving metformin and pioglitazone use in cancer patients to investigate whether these

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

treatments are potential candidates to target PAK signaling (Figure 3). Our analysis provided that pioglitazone treated patients with PAK dependent diseases had a clinical response rate of 53% (Figure 3B). It was unfortunate that data needed to calculate response rate to metformin were not available for analysis. Both pioglitazone and metformin treatment resulted is relatively few serious adverse events. However, the overwhelming majority of patients, nearly 80% of pioglitazone and 75% metformin treatment patients had mild adverse events (Figure 3B). The high number of pioglitazone associated events such as edema, and hypertension were likely due to the advanced treatment regimen used in these patients, equivalent to 45mg/day, versus the standard care at a dose equivalent to 15mg/day (Majima et al., 2006). Other studies have indicated fewer adverse events at lower doses equivalent to 7.5mg/day (Majima et al., 2006). Although gastrointestinal symptoms like constipation, diarrhea, and nausea are common side effect of metformin (Bosi, 2009), so many adverse events were unexpected and likely not attributable to dose, as patients received a standard of care equivalent of 2000mg/day. Given that metformin is the gold standard in treatment of T2DM (Bosi, 2009), the frequency of adverse events is concerning. Although not life threatening, adverse events are a serious therapeutic limitation and concern for future chemopreventive studies, as long-term patient compliance will dramatically decline if quality of life is decreased by therapy. In addition to our analysis, ten-year treatment of pioglitazone in diabetic patients was associated with increased prostate and pancreatic cancer risk, (Lewis et al., 2015), making its long-term use in chemoprevention questionable.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

We verified whether PAK signaling was even affected in these patients by pioglitazone or metformin at the molecular level (Table 6 and Figure 4), and found that pioglitazone but not metformin decreased PAK signaling. This data was in line with our analysis of signaling pathways affected by these drugs (Table 4), in that metformin induced signaling upstream of PAKs while pioglitazone rather reduced downstream signaling (Figure 2). Here pioglitazone reduced markers of proliferation including cyclinD1 and Ki67, while metformin had the opposite effect on proliferation. Long-term treatment with metformin in diabetic patients may therefore induce, not inhibit long-term cancer risk, however future studies would need to investigate this further.

Considering our clinical and molecular analysis of PAK biomarkers in pioglitazone treated patients, future studies should investigate the long term side effects associated with its treatment and whether PAK signaling in disease can be impeded early on, to block malignant transformation (Kumar et al., 2016).

This was the first study to provide a mechanistic explanation of how anti-diabetics may target PAK signaling for their potential use for chemoprevention in patients with T2DM, albeit our signaling pathway analysis included multiple pre-clinical studies, which were not of high significance. Ideally, a thorough signaling pathway analysis should use PAK1 and p-PAK1 as biomarkers in multiple human studies, however this data was unavailable for analysis due to the limitations of the current literature in regard to our highly specific question (Dammann et al., 2015). Considering the few patients which were analyzed here, the accuracy of this data is supported by its correlation between literature reports (Table 4 and Figure 2) and our analysis of PAK signaling pathways from human studies (Table 6 and Figure 4). Future studies with more patients and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

additional readouts of known PAK targets would provide a more clear analysis of whether chemoprevention in diabetes with pioglitazone is feasible long term.

Future directions

Although, not associated with diabetes, other studies of chemoprevention have shown that anti-inflammatory drugs like (aspirin in colorectal cancer), and (mesalamine in colitis associated cancer), reduce cancer-associated risk (Drew et al., 2016) (Lyakhovich & Gasche, 2010). Mesalamine, the first line treatment for chronic inflammation in ulcerative colitis (Karagozian & Burakoff, 2007), was recently established as a PAK1 inhibitor (Khare et al., 2013), and others have shown it is a PPAR-gamma ligand (Rousseaux et al., 2005); and both of these mechanisms are in line with glitazones (Dammann et al., 2015). Therefore, future directions in chemoprevention in diabetes should analyze PAK expression/ phosphorylation upon glitazone treatment and see if the effects are similar to those of mesalamine in impeding chronic inflammation in colitis associated colon cancer.

Concluding remarks

Anti-diabetics like pioglitazone rather than metformin should be utilized as a platform for further understanding the role of PAKs as a chemopreventive target in diabetes; however, before this is possible, future studies must standardize doses specific to the associated disease in order to modulate PAK signaling appropriately and minimize adverse effects. Ideal chemoprevention, like mesalamine, will block inflammation, and impede aberrant PAK signaling without altering cellular homeostasis.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

393 **Disclosure**: This manuscript was compiled as a research and literature review at
394 the Medical University of the Americas (MUA). MUA accepted the terms and
395 conditions required to further utilize this work for publication purposes.

396 **Funding**: This research did not receive any specific grant from any funding
397 agency in the public, commercial or not-for-profit sector.

398

399

400

401

402

403

404

405

406

407

408
409

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

410 **REFERENCES**
411
412 Ahn, M., Yoder, S. M., Wang, Z., Oh, E., Ramalingam, L., Tunduguru, R., &
413 Thurmond, D. C. (2016). The p21-activated kinase (PAK1) is involved in
414 diet-induced beta cell mass expansion and survival in mice and human
415 islets. *Diabetologia*, *59*(10), 2145–2155. https://doi.org/10.1007/s00125-
416 016-4042-0
417 Altomare, D. A., & Khaled, A. R. (2012). Homeostasis and the Importance for a
418 Balance Between AKT/mTOR Activity and Intracellular Signaling. *Current*
419 *Medicinal Chemistry*, *19*(22), 3748–3762.
420 https://doi.org/10.2174/092986712801661130
421 Barroso, I., Luan, J., Middelberg, R. P. S., Harding, A.-H., Franks, P. W., Jakes, R. W.,
422 … Wareham, N. J. (2003). Candidate gene association study in type 2
423 diabetes indicates a role for genes involved in beta-cell function as well as
424 insulin action. *PLoS Biology*, *1*(1), E20.
425 https://doi.org/10.1371/journal.pbio.0000020
426 Blanchard, P.-G., Festuccia, W. T., Houde, V. P., St-Pierre, P., Brule, S., Turcotte, V.,
427 … Deshaies, Y. (2012). Major involvement of mTOR in the PPARgamma-
428 induced stimulation of adipose tissue lipid uptake and fat accretion. *Journal*
429 *of Lipid Research*, *53*(6), 1117–1125. https://doi.org/10.1194/jlr.M021485
430 Bolden, A., Bernard, L., Jones, D., Akinyeke, T., & Stewart, L. V. (2012). The PPAR
431 gamma agonist troglitazone regulates erk 1/2 phosphorylation via a
432 PPAR??-independent, MEK-dependent pathway in human prostate cancer
433 cells. *PPAR Research*, *2012*. https://doi.org/10.1155/2012/929052
434 Bosi, E. (2009). Metformin--the gold standard in type 2 diabetes: what does the
435 evidence tell us? *Diabetes, Obesity & Metabolism*, *11 Suppl 2*, 3–8.
436 https://doi.org/10.1111/j.1463-1326.2008.01031.x
437 Burotto, M., & Szabo, E. (2014). PPARγ in Head and Neck Cancer Prevention. *Oral*
438 *Oncology*, *50*(10), 924–929.
439 https://doi.org/10.1016/j.oraloncology.2013.12.020
440 Campregher, C., Schmid, G., Ferk, F., Knasmuller, S., Khare, V., Kortum, B., …
441 Gasche, C. (2012). MSH3-deficiency initiates EMAST without oncogenic
442 transformation of human colon epithelial cells. *PloS One*, *7*(11), e50541.
443 https://doi.org/10.1371/journal.pone.0050541
444 Chang, S.-C., & Yang, W.-C. V. (2016). Hyperglycemia, tumorigenesis, and chronic
445 inflammation. *Critical Reviews in Oncology/Hematology*, *108*, 146–153.
446 https://doi.org/10.1016/j.critrevonc.2016.11.003
447 Chiang, Y. A., & Jin, T. (2014). p21-Activated protein kinases and their emerging
448 roles in glucose homeostasis. *American Journal of Physiology. Endocrinology*
449 *and Metabolism*, *306*(7), E707-22.
450 https://doi.org/10.1152/ajpendo.00506.2013
451 Chiang, Y. A., Shao, W., Xu, X. X., Chernoff, J., & Jin, T. (2013). P21-activated
452 protein kinase 1 (Pak1) mediates the cross talk between insulin and ??-
453 catenin on proglucagon gene expression and its ablation affects glucose
454 homeostasis in male C57BL/6 mice. *Endocrinology*, *154*(1), 77–88.
455 https://doi.org/10.1210/en.2012-1781
456 Chiang, Y. T. A., Ip, W., Shao, W., Song, Z. E., Chernoff, J., & Jin, T. (2014). Activation
457 of cAMP signaling attenuates impaired hepatic glucose disposal in aged male
458 p21-activated protein kinase-1 knockout mice. *Endocrinology*, *155*(6),

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

2122–2132. https://doi.org/10.1210/en.2013-1743

Clevers, H., Loh, K. M., & Nusse, R. (2014). Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. *Science (New York, N.Y.)*, *346*(6205), 1248012. https://doi.org/10.1126/science.1248012

Coletta, D. K., Sriwijitkamol, A., Wajcberg, E., Tantiwong, P., Li, M., Prentki, M., … Defronzo, R. A. (2009). Pioglitazone stimulates AMP-activated protein kinase signalling and increases the expression of genes involved in adiponectin signalling, mitochondrial function and fat oxidation in human skeletal muscle in vivo: a randomised trial. *Diabetologia*, *52*(4), 723–732. https://doi.org/10.1007/s00125-008-1256-9

Cuadrado, A., & Nebreda, A. R. (2010). Mechanisms and functions of p38 MAPK signalling. *The Biochemical Journal*, *429*(3), 403–417. https://doi.org/10.1042/BJ20100323

Dallaglio, K., Bruno, A., Cantelmo, A. R., Esposito, A. I., Ruggiero, L., Orecchioni, S., … Albini, A. (2014). Paradoxic effects of metformin on endothelial cells and angiogenesis. *Carcinogenesis*, *35*(5), 1055–1066. Retrieved from http://dx.doi.org/10.1093/carcin/bgu001

Dammann, K., Khare, V., & Gasche, C. (2014). Tracing PAKs from GI inflammation to cancer. *Gut*, *63*(7), 1173–1184. https://doi.org/10.1136/gutjnl-2014-306768

Dammann, K., Khare, V., Harpain, F., Lang, M., Kurtovic, A., Mesteri, I., … Gasche, C. (2015). PAK1 promotes intestinal tumor initiation. *Cancer Prevention Research (Philadelphia, Pa.)*, *8*(11), 1093–1101. https://doi.org/10.1158/1940-6207.CAPR-15-0205-T

Dammann, K., Khare, V., Lang, M., Claudel, T., Harpain, F., Granofszky, N., … Gasche, C. (2015). PAK1 modulates a PPARγ/NF-κB cascade in intestinal inflammation. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1853*(10), 2349–2360. https://doi.org/10.1016/j.bbamcr.2015.05.031

DeSantiago, J., Bare, D. J., Xiao, L., Ke, Y., Solaro, R. J., & Banach, K. (2014). p21-Activated kinase1 (Pak1) is a negative regulator of NADPH-oxidase 2 in ventricular myocytes. *Journal of Molecular and Cellular Cardiology*, *67*, 77–85. https://doi.org/10.1016/j.yjmcc.2013.12.017

DEVERY, A. M., WADEKAR, R., BOKOBZA, S. M., WEBER, A. M., JIANG, Y., & RYAN, A. J. (2015). Vascular endothelial growth factor directly stimulates tumour cell proliferation in non-small cell lung cancer. *International Journal of Oncology*, *47*(3), 849–856. https://doi.org/10.3892/ijo.2015.3082

Dodeller, F., & Schulze-Koops, H. (2006). The p38 mitogen-activated protein kinase signaling cascade in CD4 T cells. *Arthritis Research & Therapy*, *8*(2), 205. https://doi.org/10.1186/ar1905

Domenech, E., Maestre, C., Esteban-Martinez, L., Partida, D., Pascual, R., Fernandez-Miranda, G., … Malumbres, M. (2015). AMPK and PFKFB3 mediate glycolysis and survival in response to mitophagy during mitotic arrest. *Nature Cell Biology*, *17*(10), 1304–1316. https://doi.org/10.1038/ncb3231

Donath, M. Y., & Shoelson, S. E. (2011). Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol*, *11*(2), 98–107. Retrieved from http://dx.doi.org/10.1038/nri2925

Drew, D. A., Cao, Y., & Chan, A. T. (2016). Aspirin and colorectal cancer: the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

promise of precision chemoprevention. *Nat Rev Cancer*, *16*(3), 173–186. Retrieved from http://dx.doi.org/10.1038/nrc.2016.4

Drews, G., Krippeit-Drews, P., & Dufer, M. (2010). Oxidative stress and beta-cell dysfunction. *Pflugers Archiv : European Journal of Physiology*, *460*(4), 703–718. https://doi.org/10.1007/s00424-010-0862-9

Elghazi, L., Gould, A. P., Weiss, A. J., Barker, D. J., Callaghan, J., Opland, D., … Bernal-Mizrachi, E. (2012). Importance of beta-Catenin in glucose and energy homeostasis. *Scientific Reports*, *2*, 693. https://doi.org/10.1038/srep00693

Ersoy, C., Kiyici, S., Budak, F., Oral, B., Guclu, M., Duran, C., … Imamoglu, S. (2008). The effect of metformin treatment on VEGF and PAI-1 levels in obese type 2 diabetic patients. *Diabetes Research and Clinical Practice*, *81*(1), 56–60. https://doi.org/10.1016/j.diabres.2008.02.006

Essers, M. A. G., de Vries-Smits, L. M. M., Barker, N., Polderman, P. E., Burgering, B. M. T., & Korswagen, H. C. (2005). Functional interaction between beta-catenin and FOXO in oxidative stress signaling. *Science (New York, N.Y.)*, *308*(5725), 1181–1184. https://doi.org/10.1126/science.1109083

Gan, J., Zhang, Y., Ke, X., Tan, C., Ren, H., Dong, H., … Zhang, H. (2015). Dysregulation of PAK1 Is Associated with DNA Damage and Is of Prognostic Importance in Primary Esophageal Small Cell Carcinoma. *International Journal of Molecular Sciences*, *16*(6), 12035–12050. https://doi.org/10.3390/ijms160612035

Gautam, S., Ishrat, N., Singh, R., Narender, T., & Srivastava, A. K. (2015). Aegeline from Aegle marmelos stimulates glucose transport via Akt and Rac1 signaling, and contributes to a cytoskeletal rearrangement through PI3K/Rac1. *European Journal of Pharmacology*, *762*, 419–429. https://doi.org/10.1016/j.ejphar.2015.05.021

Girnita, L., Girnita, A., & Larsson, O. (2003). Mdm2-dependent ubiquitination and degradation of the insulin-like growth factor 1 receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(14), 8247–8252. https://doi.org/10.1073/pnas.1431613100

Girnun, G. D., Smith, W. M., Drori, S., Sarraf, P., Mueller, E., Eng, C., … Spiegelman, B. M. (2002). APC-dependent suppression of colon carcinogenesis by PPARgamma. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(21), 13771–13776. https://doi.org/10.1073/pnas.162480299

Grommes, C., Karlo, J. C., Caprariello, A., Blankenship, D., Dechant, A., & Landreth, G. E. (2013). The PPARgamma agonist pioglitazone crosses the blood-brain barrier and reduces tumor growth in a human xenograft model. *Cancer Chemotherapy and Pharmacology*, *71*(4), 929–936. https://doi.org/10.1007/s00280-013-2084-2

Gu, S., Kounenidakis, M., Schmidt, E.-M., Deshpande, D., Alkahtani, S., Alarifi, S., … Stournaras, C. (2013). Rapid activation of FAK/mTOR/p70S6K/PAK1-signaling controls the early testosterone-induced actin reorganization in colon cancer cells. *Cellular Signalling*, *25*(1), 66–73.

Hou, Y., Moreau, F., & Chadee, K. (2012). PPARgamma is an E3 ligase that induces the degradation of NFkappaB/p65. *Nature Communications*, *3*, 1300. https://doi.org/10.1038/ncomms2270

Huynh, N., Liu, K. H., Baldwin, G. S., & He, H. (2010). P21-activated kinase 1

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

stimulates colon cancer cell growth and migration/invasion via ERK- and AKT-dependent pathways. *Biochimica et Biophysica Acta*, *1803*(9), 1106–1113. https://doi.org/10.1016/j.bbamcr.2010.05.007

Ijuin, T., & Takenawa, T. (2012). Regulation of Insulin Signaling by the Phosphatidylinositol 3,4,5-Triphosphate Phosphatase SKIP through the Scaffolding Function of Pak1. *Molecular and Cellular Biology*, *32*(17), 3570–3584. https://doi.org/10.1128/MCB.00636-12

Ishida, H., Li, K., Yi, M., & Lemon, S. M. (2007). p21-activated kinase 1 is activated through the mammalian target of rapamycin/p70 S6 kinase pathway and regulates the replication of hepatitis C virus in human hepatoma cells. *The Journal of Biological Chemistry*, *282*(16), 11836–11848. https://doi.org/10.1074/jbc.M610106200

Isoda, K., Young, J. L., Zirlik, A., MacFarlane, L. A., Tsuboi, N., Gerdes, N., … Libby, P. (2006). Metformin inhibits proinflammatory responses and nuclear factor-kappaB in human vascular wall cells. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *26*(3), 611–617. https://doi.org/10.1161/01.ATV.0000201938.78044.75

Ito, M., Nishiyama, H., Kawanishi, H., Matsui, S., Guilford, P., Reeve, A., & Ogawa, O. (2007). P21-activated kinase 1: a new molecular marker for intravesical recurrence after transurethral resection of bladder cancer. *The Journal of Urology*, *178*(3 Pt 1), 1073–1079. https://doi.org/10.1016/j.juro.2007.05.012

Karagozian, R., & Burakoff, R. (2007). The role of mesalamine in the treatment of ulcerative colitis. *Therapeutics and Clinical Risk Management*, *3*(5), 893–903.

Karar, J., & Maity, A. (2011). PI3K/AKT/mTOR Pathway in Angiogenesis. *Frontiers in Molecular Neuroscience*, *4*, 51. https://doi.org/10.3389/fnmol.2011.00051

Khare, V., Dammann, K., Asboth, M., Krnjic, A., Jambrich, M., & Gasche, C. (2015). Overexpression of PAK1 promotes cell survival in inflammatory bowel diseases and colitis-associated cancer. *Inflammatory Bowel Diseases*, *21*(2), 287–296. https://doi.org/10.1097/MIB.0000000000000281

Khare, V., Lyakhovich, A., Dammann, K., Lang, M., Borgmann, M., Tichy, B., … Gasche, C. (2013). Mesalamine modulates intercellular adhesion through inhibition of p-21 activated kinase-1. *Biochemical Pharmacology*, *85*(2), 234–244. https://doi.org/10.1016/j.bcp.2012.10.026

Kong, D., Dagon, Y., Campbell, J. N., Guo, Y., Yang, Z., Yi, X., … Lowell, B. B. (2016). A Postsynaptic AMPK-->p21-Activated Kinase Pathway Drives Fasting-Induced Synaptic Plasticity in AgRP Neurons. *Neuron*, *91*(1), 25–33. https://doi.org/10.1016/j.neuron.2016.05.025

Kotlinowski, J., & Jozkowicz, A. (2016). PPAR Gamma and Angiogenesis: Endothelial Cells Perspective. *Journal of Diabetes Research*, *2016*, 8492353. https://doi.org/10.1155/2016/8492353

Kumar, A., Al-Sammarraie, N., DiPette, D. J., & Singh, U. S. (2014). Metformin impairs Rho GTPase signaling to induce apoptosis in neuroblastoma cells and inhibits growth of tumors in the xenograft mouse model of neuroblastoma. *Oncotarget.*, *5*(1949–2553 (Electronic)), 11709–11722. https://doi.org/10.18632/oncotarget.2606

Kumar, R., & Li, D.-Q. (2016). PAKs in Human Cancer Progression: From Inception to Cancer Therapeutic to Future Oncobiology. *Advances in Cancer*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

606 *Research*, *130*, 137–209. https://doi.org/10.1016/bs.acr.2016.01.002
607 Kung, C.-P., & Murphy, M. E. (2016). The role of the p53 tumor suppressor in
608 metabolism and diabetes. *The Journal of Endocrinology*, *231*(2), R61–R75.
609 https://doi.org/10.1530/JOE-16-0324
610 Lewis, J. D., Habel, L. A., Quesenberry, C. P., Strom, B. L., Peng, T., Hedderson, M.
611 M., … Ferrara, A. (2015). Pioglitazone Use and Risk of Bladder Cancer and
612 Other Common Cancers in Persons With Diabetes. *JAMA*, *314*(3), 265–277.
613 https://doi.org/10.1001/jama.2015.7996
614 Li, R., Debreceni, B., Jia, B., Gao, Y., Tigyi, G., & Zheng, Y. (1999). Localization of the
615 PAK1-, WASP-, and IQGAP1-specifying regions of Cdc42. *The Journal of*
616 *Biological Chemistry*, *274*(42), 29648–29654.
617 Li, X., Cheng, K. K. Y., Liu, Z., Yang, J.-K., Wang, B., Jiang, X., … Xu, A. (2016). The
618 MDM2-p53-pyruvate carboxylase signalling axis couples mitochondrial
619 metabolism to glucose-stimulated insulin secretion in pancreatic beta-cells.
620 *Nature Communications*, *7*, 11740. https://doi.org/10.1038/ncomms11740
621 Li, X., Lee, C., Tang, Z., Zhang, F., Arjunan, P., Li, Y., … Dong, L. (2009). VEGF-B: a
622 survival, or an angiogenic factor? *Cell Adhesion & Migration*, *3*(4), 322–327.
623 Liou, G.-Y., & Storz, P. (2010). Reactive oxygen species in cancer. *Free Radical*
624 *Research*, *44*(5). https://doi.org/10.3109/10715761003667554
625 Liu, T., Li, Y., Gu, H., Zhu, G., Li, J., Cao, L., & Li, F. (2013). P21-Activated kinase 6
626 (PAK6) inhibits prostate cancer growth via phosphorylation of androgen
627 receptor and tumorigenic E3 ligase murine double minute-2 (MDM2).
628 *Journal of Biological Chemistry*, *288*(5), 3359–3369.
629 https://doi.org/10.1074/jbc.M112.384289
630 Liu, Y., Wang, S., Dong, Q.-Z., Jiang, G.-Y., Han, Y., Wang, L., & Wang, E.-H. (2016).
631 The P21-activated kinase expression pattern is different in non-small cell
632 lung cancer and affects lung cancer cell sensitivity to epidermal growth
633 factor receptor tyrosine kinase inhibitors. *Medical Oncology (Northwood,*
634 *London, England)*, *33*(3), 22. https://doi.org/10.1007/s12032-016-0735-y
635 Lu, D., & Carson, D. A. (2010). Repression of beta-catenin signaling by PPAR
636 gamma ligands. *European Journal of Pharmacology*, *636*(1–3), 198–202.
637 https://doi.org/10.1016/j.ejphar.2010.03.010
638 Lu, W., Wang, X., Liu, J., He, Y., Liang, Z., Xia, Z., … Liang, S. (2016). Downregulation
639 of ARHGDIA contributes to human glioma progression through activation of
640 Rho GTPase signaling pathway. *Tumour Biology : The Journal of the*
641 *International Society for Oncodevelopmental Biology and Medicine*.
642 https://doi.org/10.1007/s13277-016-5374-6
643 Lyakhovich, A., & Gasche, C. (2010). Systematic review: molecular
644 chemoprevention of colorectal malignancy by mesalazine. *Alimentary*
645 *Pharmacology & Therapeutics*, *31*(2), 202–209.
646 https://doi.org/10.1111/j.1365-2036.2009.04195.x
647 Ma, J., Xue, Y., Liu, W., Yue, C., Bi, F., Xu, J., … Chen, Y. (2013). Role of activated
648 Rac1/Cdc42 in mediating endothelial cell proliferation and tumor
649 angiogenesis in breast cancer. *PloS One*, *8*(6), e66275.
650 https://doi.org/10.1371/journal.pone.0066275
651 Majima, T., Komatsu, Y., Doi, K., Shigemoto, M., Takagi, C., Fukao, A., … Nakao, K.
652 (2006). Safety and efficacy of low-dose pioglitazone (7.5 mg/day) vs.
653 standard-dose pioglitazone (15 mg/day) in Japanese women with type 2
654 diabetes mellitus. *Endocrine Journal*, *53*(3), 325–330.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

Manchester, J., Kong, X., Lowry, O. H., & Lawrence, J. C. (1994). Ras signaling in the activation of glucose transport by insulin. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(11), 4644–4648.

Marselli, L., Thorne, J., Dahiya, S., Sgroi, D. C., Sharma, A., Bonner-Weir, S., … Weir, G. C. (2010). Gene Expression Profiles of Beta-Cell Enriched Tissue Obtained by Laser Capture Microdissection from Subjects with Type 2 Diabetes. *PLoS ONE*, *5*(7). https://doi.org/10.1371/journal.pone.0011499

Mauro, C., Leow, S. C., Anso, E., Rocha, S., Thotakura, A. K., Tornatore, L., … Franzoso, G. (2011). NF-kappaB controls energy homeostasis and metabolic adaptation by upregulating mitochondrial respiration. *Nature Cell Biology*, *13*(10), 1272–1279. https://doi.org/10.1038/ncb2324

Mazumdar, A., & Kumar, R. (2003). Estrogen regulation of Pak1 and FKHR pathways in breast cancer cells. *FEBS Letters*, *535*(1–3), 6–10.

McCarthy, M. I. (2010). Genomics, type 2 diabetes, and obesity. *The New England Journal of Medicine*, *363*(24), 2339–2350. https://doi.org/10.1056/NEJMra0906948

Menard, R. E., & Mattingly, R. R. (2003). Cell surface receptors activate p21-activated kinase 1 via multiple Ras and PI3-kinase-dependent pathways. *Cellular Signalling*, *15*(12), 1099–1109.

Moreno-Navarrete, J. M., Petrov, P., Serrano, M., Ortega, F., Garcia-Ruiz, E., Oliver, P., … Fernandez-Real, J. M. (2013). Decreased RB1 mRNA, protein, and activity reflect obesity-induced altered adipogenic capacity in human adipose tissue. *Diabetes*, *62*(6), 1923–1931. https://doi.org/10.2337/db12-0977

Motoshima, H., Goldstein, B. J., Igata, M., & Araki, E. (2006). AMPK and cell proliferation – AMPK as a therapeutic target for atherosclerosis and cancer. *The Journal of Physiology*, *574*(Pt 1), 63–71. https://doi.org/10.1113/jphysiol.2006.108324

Murray, B. W., Guo, C., Piraino, J., Westwick, J. K., Zhang, C., Lamerdin, J., … Smeal, T. (2010). Small-molecule p21-activated kinase inhibitor PF-3758309 is a potent inhibitor of oncogenic signaling and tumor growth. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(20), 9446–9451. https://doi.org/10.1073/pnas.0911863107

Nair, V., Sreevalsan, S., Basha, R., Abdelrahim, M., Abudayyeh, A., Rodrigues Hoffman, A., & Safe, S. (2014). Mechanism of metformin-dependent inhibition of mammalian target of rapamycin (mTOR) and Ras activity in pancreatic cancer: role of specificity protein (Sp) transcription factors. *The Journal of Biological Chemistry*, *289*(40), 27692–27701. https://doi.org/10.1074/jbc.M114.592576

Neumann, M., Foryst-Ludwig, A., Klar, S., Schweitzer, K., & Naumann, M. (2006). The PAK1 autoregulatory domain is required for interaction with NIK in Helicobacter pylori-induced NF-kappaB activation. *Biological Chemistry*, *387*(1), 79–86. https://doi.org/10.1515/BC.2006.011

Nola, S., Sebbagh, M., Marchetto, S., Osmani, N., Nourry, C., Audebert, S., … Santoni, M.-J. (2008). Scrib regulates PAK activity during the cell migration process. *Human Molecular Genetics*, *17*(22), 3552–3565. https://doi.org/10.1093/hmg/ddn248

Okami, N., Narasimhan, P., Yoshioka, H., Sakata, H., Kim, G. S., Jung, J. E., … Chan, P. H. (2013). Prevention of JNK phosphorylation as a mechanism for

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

rosiglitazone in neuroprotection after transient cerebral ischemia: activation of dual specificity phosphatase. *Journal of Cerebral Blood Flow & Metabolism*, *33*(1), 106–114. https://doi.org/10.1038/jcbfm.2012.138

Ouchi, N., Shibata, R., & Walsh, K. (2005). AMP-activated protein kinase signaling stimulates VEGF expression and angiogenesis in skeletal muscle. *Circulation Research*, *96*(8), 838–846. https://doi.org/10.1161/01.RES.0000163633.10240.3b

Park, M.-H., Kim, D.-J., You, S.-T., Lee, C.-S., Kim, H. K., Park, S. M., … Kim, E.-G. (2012). Phosphorylation of beta-catenin at serine 663 regulates its transcriptional activity. *Biochemical and Biophysical Research Communications*, *419*(3), 543–549. https://doi.org/10.1016/j.bbrc.2012.02.056

Park, S.-Y., Lee, J. H., Ha, M., Nam, J.-W., & Kim, V. N. (2009). miR-29 miRNAs activate p53 by targeting p85 alpha and CDC42. *Nature Structural & Molecular Biology*, *16*(1), 23–29. https://doi.org/10.1038/nsmb.1533

Parvathy, M., Sreeja, S., Kumar, R., & Pillai, M. R. (2016). Potential role of p21 Activated Kinase 1 (PAK1) in the invasion and motility of oral cancer cells. *BMC Cancer*, *16 Suppl 1*, 293. https://doi.org/10.1186/s12885-016-2263-8

Picard, F., & Auwerx, J. (2002). PPAR(gamma) and glucose homeostasis. *Annual Review of Nutrition*, *22*, 167–197. https://doi.org/10.1146/annurev.nutr.22.010402.102808

Puto, L. A., Pestonjamasp, K., King, C. C., & Bokoch, G. M. (2003). p21-activated kinase 1 (PAK1) interacts with the Grb2 adapter protein to couple to growth factor signaling. *The Journal of Biological Chemistry*, *278*(11), 9388–9393. https://doi.org/10.1074/jbc.M208414200

Qian, X., Li, J., Ding, J., Wang, Z., Duan, L., & Hu, G. (2008). Glibenclamide exerts an antitumor activity through reactive oxygen species-c-jun NH2-terminal kinase pathway in human gastric cancer cell line MGC-803. *Biochemical Pharmacology*, *76*(12), 1705–1715. https://doi.org/10.1016/j.bcp.2008.09.009

Raut, S. K., Kumar, A., Singh, G. B., Nahar, U., Sharma, V., Mittal, A., … Khullar, M. (2015). miR-30c Mediates Upregulation of Cdc42 and Pak1 in Diabetic Cardiomyopathy. *Cardiovascular Therapeutics*, *33*(3), 89–97. https://doi.org/10.1111/1755-5922.12113

Reuter, S., Gupta, S. C., Chaturvedi, M. M., & Aggarwal, B. B. (2010). Oxidative stress, inflammation, and cancer: How are they linked? *Free Radical Biology & Medicine*, *49*(11), 1603–1616. https://doi.org/10.1016/j.freeradbiomed.2010.09.006

Rousseaux, C., Lefebvre, B., Dubuquoy, L., Lefebvre, P., Romano, O., Auwerx, J., … Desreumaux, P. (2005). Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor-gamma. *The Journal of Experimental Medicine*, *201*(8), 1205–1215. https://doi.org/10.1084/jem.20041948

Salminen, A., Hyttinen, J. M. T., & Kaarniranta, K. (2011). AMP-activated protein kinase inhibits NF-κB signaling and inflammation: impact on healthspan and lifespan. *Journal of Molecular Medicine (Berlin, Germany)*, *89*(7), 667–676. https://doi.org/10.1007/s00109-011-0748-0

Salomone, S. (2011). Pleiotropic Effects of Glitazones: A Double Edge Sword? . *Frontiers in Pharmacology* . Retrieved from

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

753 http://journal.frontiersin.org/article/10.3389/fphar.2011.00014
754 San, Y.-Z., Liu, Y., Zhang, Y., Shi, P.-P., & Zhu, Y.-L. (2015). Peroxisome
755 proliferator-activated receptor-gamma agonist inhibits the mammalian
756 target of rapamycin signaling pathway and has a protective effect in a rat
757 model of status epilepticus. *Molecular Medicine Reports*, *12*(2), 1877–1883.
758 https://doi.org/10.3892/mmr.2015.3641
759 Shalom-Barak, T., & Knaus, U. G. (2002). A p21-activated kinase-controlled
760 metabolic switch up-regulates phagocyte NADPH oxidase. *Journal of*
761 *Biological Chemistry*, *277*(43), 40659–40665.
762 https://doi.org/10.1074/jbc.M206650200
763 Shi, Y., Nikulenkov, F., Zawacka-Pankau, J., Li, H., Gabdoulline, R., Xu, J., …
764 Selivanova, G. (2014). ROS-dependent activation of JNK converts p53 into an
765 efficient inhibitor of oncogenes leading to robust apoptosis. *Cell Death and*
766 *Differentiation*, *21*(4), 612–623. https://doi.org/10.1038/cdd.2013.186
767 Shin, Y. J., Kim, E. H., Roy, A., & Kim, J.-H. (2013). Evidence for a novel mechanism
768 of the PAK1 interaction with the Rho-GTPases Cdc42 and Rac. *PloS One*,
769 *8*(8), e71495. https://doi.org/10.1371/journal.pone.0071495
770 Song, B., Wang, W., Zheng, Y., Yang, J., & Xu, Z. (2015). P21-activated kinase 1 and
771 4 were associated with colorectal cancer metastasis and infiltration. *The*
772 *Journal of Surgical Research*, *196*(1), 130–135.
773 https://doi.org/10.1016/j.jss.2015.02.035
774 Sozen, B., Ozturk, S., Yaba, A., & Demir, N. (2015). The p38 MAPK signalling
775 pathway is required for glucose metabolism, lineage specification and
776 embryo survival during mouse preimplantation development. *Mechanisms*
777 *of Development*, *138 Pt 3*, 375–398.
778 https://doi.org/10.1016/j.mod.2015.05.002
779 Sylow, L., Kleinert, M., Pehmøller, C., Prats, C., Chiu, T. T., Klip, A., … Jensen, T. E.
780 (2014). Akt and Rac1 signaling are jointly required for insulin-stimulated
781 glucose uptake in skeletal muscle and downregulated in insulin resistance.
782 *Cellular Signalling*, *26*(2), 323–331.
783 https://doi.org/10.1016/j.cellsig.2013.11.007
784 Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas,
785 J., … von Mering, C. (2015). STRING v10: protein-protein interaction
786 networks, integrated over the tree of life. *Nucleic Acids Research*,
787 *43*(Database issue), D447-52. https://doi.org/10.1093/nar/gku1003
788 Taneera, J., Fadista, J., Ahlqvist, E., Zhang, M., Wierup, N., Renstrom, E., & Groop, L.
789 (2013). Expression profiling of cell cycle genes in human pancreatic islets
790 with and without type 2 diabetes. *Molecular and Cellular Endocrinology*,
791 *375*(1–2), 35–42. https://doi.org/10.1016/j.mce.2013.05.003
792 Tomar, A., & Schlaepfer, D. D. (2010). A PAK-activated linker for EGFR and FAK.
793 *Developmental Cell*, *18*(2), 170–172.
794 https://doi.org/10.1016/j.devcel.2010.01.013
795 Viollet, B., Lantier, L., Devin-Leclerc, J., Hébrard, S., Amouyal, C., Mounier, R., …
796 Andreelli, F. (2009). Targeting the AMPK pathway for the treatment of Type
797 2 diabetes. *Frontiers in Bioscience (Landmark Edition)*, *14*, 3380–3400.
798 Wagner, K., Hemminki, K., Grzybowska, E., Klaes, R., Butkiewicz, D., Pamula, J., …
799 Forsti, A. (2004). The insulin-like growth factor-1 pathway mediator genes:
800 SHC1 Met300Val shows a protective effect in breast cancer. *Carcinogenesis*,
801 *25*(12), 2473–2478. https://doi.org/10.1093/carcin/bgh263

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 August 2018 doi:10.20944/preprints201808.0403.v1

802 Wang, P.-S., Chou, F.-S., Bloomston, M., Vonau, M. S., Saji, M., Espinosa, A., &
803 Pinzone, J. J. (2009). Thiazolidinediones downregulate Wnt/beta-catenin
804 signaling via multiple mechanisms in breast cancer cells. *The Journal of*
805 *Surgical Research*, *153*(2), 210–216.
806 https://doi.org/10.1016/j.jss.2008.05.032
807 Wang, Z., Oh, E., & Thurmond, D. C. (2007). Glucose-stimulated Cdc42 signaling is
808 essential for the second phase of insulin secretion. *Journal of Biological*
809 *Chemistry*, *282*(13), 9536–9546. https://doi.org/10.1074/jbc.M610553200
810 Wang, Z., & Thurmond, D. C. (2010). Differential phosphorylation of RhoGDI
811 mediates the distinct cycling of Cdc42 and Rac1 to regulate second-phase
812 insulin secretion. *The Journal of Biological Chemistry*, *285*(9), 6186–6197.
813 https://doi.org/10.1074/jbc.M109.072421
814 Wei, W.-Y., Ma, Z.-G., Xu, S.-C., Zhang, N., & Tang, Q.-Z. (2016). Pioglitazone
815 Protected against Cardiac Hypertrophy via Inhibiting AKT/GSK3beta and
816 MAPK Signaling Pathways. *PPAR Research*, *2016*, 9174190.
817 https://doi.org/10.1155/2016/9174190
818 Wu, N., Gu, C., Gu, H., Hu, H., Han, Y., & Li, Q. (2011). Metformin induces apoptosis
819 of lung cancer cells through activating JNK/p38 MAPK pathway and
820 GADD153. *Neoplasma*, *58*(6), 482–490.
821 Xu, H., Zhou, Y., Liu, Y., Ping, J., Shou, Q., Chen, F., & Ruo, R. (2016). Metformin
822 improves hepatic IRS2/PI3K/Akt signaling in insulin-resistant rats of NASH
823 and cirrhosis. *The Journal of Endocrinology*, *229*(2), 133–144.
824 https://doi.org/10.1530/JOE-15-0409
825 You, G. Y., Lee, J. O., Kim, J. H., Kim, N., Lee, S. K., Moon, J. W., … Kim, H. S. (2013).
826 Tiam-1, a GEF for Rac1, plays a critical role in metformin-mediated glucose
827 uptake in C2C12 cells. *Cellular Signalling*, *25*(12), 2558–2565.
828 https://doi.org/10.1016/j.cellsig.2013.08.018
829 Yuan, X., Zhang, Z., Gong, K., Zhao, P., Qin, J., & Liu, N. (2011). Inhibition of
830 reactive oxygen species/extracellular signal-regulated kinases pathway by
831 pioglitazone attenuates advanced glycation end products-induced
832 proliferation of vascular smooth muscle cells in rats. *Biological &*
833 *Pharmaceutical Bulletin*, *34*(5), 618–623.
834 Zhang, W., Thompson, B. J., Hietakangas, V., & Cohen, S. M. (2011). MAPK/ERK
835 signaling regulates insulin sensitivity to control glucose metabolism in
836 Drosophila. *PLoS Genetics*, *7*(12), e1002429.
837 https://doi.org/10.1371/journal.pgen.1002429
838 Zhu, G., Wang, Y., Huang, B., Liang, J., Ding, Y., Xu, A., & Wu, W. (2012). A
839 Rac1/PAK1 cascade controls beta-catenin activation in colon cancer cells.
840 *Oncogene*, *31*(8), 1001–1012. https://doi.org/10.1038/onc.2011.294
841