Ten Best Publications: **Full length Research Articles as Corresponding Authors:**

1) **MicroRNA-99a mimics inhibit M1 macrophage phenotype and adipose tissue inflammation by targeting TNFα.**

Jaiswal A, Reddy SS, Maurya M, Maurya P, Barthwal MK. **Cell Mol. Immunol**. 2019 May;16 (5):495-507. (**I.F 24.1**)

In human adipose tissue and obesity, miR-99a expression is negatively correlated with inflammation. Therefore, the present study investigated the role of miR-99a in macrophage phenotype activation and adipose tissue inflammation. M2 BMDMs showed a significant increase in miR-99a expression when compared to the M0 and M1 phenotypes. Phenotype-switching experiments established an association between upregulated miR-99a expression and the M2 phenotype. Overexpression of miR-99a prevented M1 phenotype activation and attenuated bactericidal activity. Likewise, knockdown of miR-99a abolished M2 phenotype activation. By means of in silico target prediction tools and a luciferase reporter assay, TNFα was identified as a direct target of miR-99a. Knockdown of TNFα recapitulated the effect of miR-99a overexpression in M1 BMDMs. In a db/db mice model, miR-99a expression was reduced in eWAT and F4/80+ ATMs. Systemic overexpression of miR-99a in db/db mice attenuated adipocyte hypertrophy with increased CD301 and reduced CD86 immunostaining. Flow cytometry analysis also showed an increased M2 and a reduced M1 macrophage population. Mimics of miR-99a also improved the diabetic dyslipidemia and insulin signaling in eWAT and liver, with an attenuated expression of gluconeogenesis and cholesterol metabolism genes in the liver. Furthermore, adoptive transfer of miR-99a-overexpressing macrophages in the db/db mice recapitulated in vivo miR-99a mimic effects with increased M2 and reduced M1 macrophage populations and improved systemic glucose, insulin sensitivity, and insulin signaling in the eWAT and liver.

Therefore, the present study demonstrates that miR-99a mimics can regulate macrophage M1 phenotype activation by targeting TNFα. miR-99a therapeutics in diabetic mice reduces the adipose tissue inflammation and improves insulin sensitivity.

2) **The IRAK-ERK-p67phox-Nox-2 axis mediates TLR4, 2-induced ROS production for IL-1β transcription and processing in monocytes.**

Singh A, Singh V, Tiwari RL, Chandra T, Kumar A, Dikshit M, Barthwal MK. **Cell Mol Immunol**. 2016 Nov;13(6):745-763. (**I.F 24.1**)

In monocytic cells, Toll-like receptor 4 (TLR4)- and TLR2-induced reactive oxygen species (ROS) cause oxidative stress and inflammatory response; however, the mechanism is not well understood. The present study investigated the role of interleukin-1 receptor-associated kinase (IRAK), extracellular signal-regulated kinase (ERK), p67phox and Nox-2 in TLR4- and TLR2-induced ROS generation during interleukin-1 beta (IL-1β) transcription, processing, and secretion. An IRAK1/4 inhibitor, U0126, PD98059, an NADPH oxidase inhibitor (diphenyleneiodonium (DPI)), and a free radical scavenger (N-acetyl cysteine (NAC))-attenuated TLR4 (lipopolysaccharide (LPS))- and TLR2 (Pam3csk4)-induced ROS generation and IL-1β production in THP-1 and primary human monocytes. An IRAK1/4 inhibitor and siRNA-attenuated LPS- and Pam3csk4-induced ERK-IRAK1 association and ERK phosphorylation and activity. LPS and Pam3csk4 also induced IRAK1/4-, ERK- and ROS-dependent activation of activator protein-1 (AP-1), IL-1β transcription, and IL-1β processing because significant inhibition in AP-1 activity, IL-1β transcription, Pro- and mature IL-β expression, and caspase-1 activity was observed with PD98059, U0126, DPI, NAC, an IRAK1/4 inhibitor, tanshinone IIa, and IRAK1 siRNA treatment. IRAK-dependent ERK-p67phox interaction, p67phox translocation, and p67phox-Nox-2 interaction were observed. Nox-2 siRNA significantly reduced secreted IL-1β, IL-1β transcript, pro- and

mature IL-1β expression, and caspase-1 activity indicating a role for Nox-2 in LPS- and Pam3csk4-induced IL-1β production, transcription, and processing.

In the present study, we demonstrate that the TLR4- and TLR2-induced IRAK-ERK pathway cross-talks with p67phox-Nox-2 for ROS generation, thus regulating IL-1β transcription and processing in monocytic cells.

3) **Involvement of interleukin-1 receptor-associated kinase-1 in vascular smooth muscle cell proliferation and neointimal formation after rat carotid injury.**

Jain M, Singh A, Singh V, Barthwal MK. **Arterioscler Thromb Vasc Biol.** 2015 Jun;35(6):1445-55. (**I.F 8.7**)

Reduced frequency of atherosclerotic plaques is observed in interleukin-1 receptor-associated kinase-1 (IRAK1)-deficient mice; however, the underlying mechanism is not clear. Therefore, this study investigate the role of IRAK1 in vascular smooth muscle cell proliferation and neointimal hyperplasia. Stimulation of rat primary vascular smooth muscle cells with fetal bovine serum (10%) or platelet-derived growth factor-BB (20 ng/mL) for 15 minutes to 24 hours induced a time-dependent increase in IRAK1 and extracellular signal-regulated kinase (ERK) activation, proliferating cell nuclear antigen upregulation and p27Kip1 downregulation as assessed by Western blotting. Inhibitors of ERK pathway (U0126, 10 μmol/L), IRAK (IRAK1/4, 3 μmol/L), protein kinase C (PKC; Ro-31-8220, 1 μmol/L), siRNA of toll-like receptor-4 (200 nmol/L), and PKC-ε (200 nmol/L) significantly attenuated these changes. Platelet-derived growth factor induced endogenous IRAK-ERK-PKC-ε association in a toll-like receptor-4 and PKC-ε-dependent manner. A time-dependent increase in IRAK1 and ERK activation was observed after 15 minutes, 30 minutes, 1 hour, 6 hours, 12 hours, and 24 hours of carotid balloon injury in rats. Balloon injury induced endogenous IRAK-ERK-PKC-ε interaction. Perivascular application of IRAK1/4 inhibitor (100 μmol/L), U0126 (100 μmol/L), and IRAK1 siRNA (220 and 360 nmol/L) in pluronic gel abrogated balloon injury-induced ERK phosphorylation, activation, and p27Kip1 downregulation. Hematoxylin and eosin staining and immunohistochemistry of proliferating cell nuclear antigen and smooth muscle actin demonstrated that balloon injury-induced intimal thickening and neointimal vascular smooth muscle cell proliferation were significantly abrogated in the presence of IRAK1/4 inhibitor, IRAK1 siRNA, and U0126.

Therefore, it can be concluded that IRAK1 mediates vascular smooth muscle cell proliferation and neo-intimal hyperplasia by regulating PKC-ε-IRAK1-ERK axis.

4) **Macrophage p47 phox regulates pressure overload-induced left ventricular remodeling by modulating IL-4/STAT6/PPARγ signalling.**

Reddy SS, Agarwal H, Jaiswal A, Jagavelu K, Dikshit M, Barthwal MK. **Free Radic Biol Med**. 2021 May 20;168:168-179. (**I.F. 7.4**)

NADPH oxidase (Nox) mediates ROS production and contributes to cardiac remodelling. However, macrophage p47phox, a Nox subunit regulating cardiac remodelling, is unclear. We aimed to investigate the role of macrophage p47phox in hypertensive cardiac remodelling. Pressure-overload induced by Angiotensin II (AngII) for two weeks in young adult male p47phox deficient (KO) mice showed aggravated cardiac dysfunction and hypertrophy as indicated from echocardiographic and histological studies in comparison with wild-type littermates (WT). Additionally, LV of AngII-infused KO mice showed augmented interstitial fibrosis, collagen deposition and, myo-fibroblasts compared to AngII-infused WT mice. Moreover, these changes in AngII-infused KO mice correlated well with the gene analysis of hypertrophic and fibrotic markers. Similar results were also found in the transverse aortic constriction model. Further, AngII-infused KO mice showed elevated circulating immunokines and increased LV leukocytes infiltration and CD206+ macrophages compared to AngII-infused WT

mice. Likewise, LV of AngII-infused KO mice showed upregulated mRNA expression of anti-inflammatory/pro-fibrotic M2 macrophage markers (Ym1, Arg-1) compared to AngII-infused WT mice. AngII and IL-4 treated bone marrow-derived macrophages (BMDMs) from KO mice showed upregulated M2 macrophage markers and STAT6 phosphorylation (Y641) compared to AngII and IL-4 treated WT BMDMs. These alterations were at least partly mediated by macrophage as bone marrow transplantation from KO mice into WT mice aggravated cardiac remodeling. Mechanistically, AngII-infused KO mice showed hyperactivated IL-4/STAT6/PPARγ signaling and downregulated SOCS3 expression compared to AngII-infused WT mice. Our studies show that macrophage p47phox limits anti-inflammatory signaling and extracellular matrix remodeling in response to pressure-overload.

5) **Galectin-3 S-glutathionylation regulates its effect on adipocyte insulin signaling**.

Maurya M, Jaiswal A, Gupta S, Ali W, Gaikwad AN, Dikshit M, Barthwal MK. **Biochim Biophys Acta Mol Cell Res.** 2022 Jun;1869(6):119234. (**I.F. 5.1**).

Protein-S-glutathionylation promotes redox signaling in physiological and oxidative distress conditions. Galectin-3 (Gal-3) promotes insulin resistance by down-regulating adipocyte insulin signaling, however, its S-glutathionylation and significance is not known. In this context, we report reversible S-glutathionylation of Gal-3. Site-directed mutagenesis established Gal-3 Cys187 as the putative S-glutathionylation site. Glutathionylated Gal-3 prevents Gal-3(WT)-Insulin Receptor interaction and facilitates insulin-induced murine adipocyte p-IRS1(tyr895) and p-AKT(ser473) signaling and glucose uptake in a Gal-3 Cys187 glutathionylation dependent manner in murine adipocytes, as assessed by Western blotting and 2-NBDG uptake assay respectively. Pre-glutathionylated Gal-3 at Cys187 resisted irreversible oxidation by H2O2. M2 macrophages showed enhanced Gal-3 S-glutathionylation when compared to M1 phenotype. Serum and stromal vascular fraction (SVF) isolated from control mice showed increased Gal-3 S-glutathionylation as compared to db/db mice. A significant increase in Gal-3 S-glutathionylation was observed in metformin-treated db/db mice when compared to db/db mice alone. Similar to murine, enhanced Gal-3 S-glutathionylation is observed in primary human monocyte derived M2 macrophages when compared to the M1 macrophage phenotype and Gal-3 regulates primary human adipocyte insulin signaling in a glutathionylation dependent manner. Collectively, we identified Gal-3 S-glutathionylation as a protective phenomenon, which relieves its inhibitory effect on adipocyte insulin signaling.

6) **Curcuma oil ameliorates hyperlipidaemia and associated deleterious effects in golden Syrian hamsters**.

Singh V, Rana M, Jain M, Singh N, Naqvi A, Malasoni R, Dwivedi AK, Dikshit M, Barthwal MK. **Br J Nutr.** 2013 Aug 28;110(3):437-46. (**I.F: 3.6**)

Essential oil components from turmeric (Curcuma longa L.) are documented for neuroprotective, anti-cancer, anti-thrombotic and antioxidant effects. The present study aimed to investigate the disease-modifying potential of curcuma oil (C. oil), a lipophilic component from C. longa L., in hyperlipidaemic hamsters. Male golden Syrian hamsters were fed a chow or high-cholesterol (HC) and fat-rich diet with or without C. oil (30, 100 and 300 mg/kg) for 28 d. In HC diet-fed hamsters, C. oil significantly reduced plasma total cholesterol, LDL-cholesterol and TAG, and increased HDL-cholesterol when compared with the HC group. Similar group comparisons showed that C. oil treatment reduced hepatic cholesterol and oxidative stress, and improved liver function. Hyperlipidaemia-induced platelet activation, vascular dysfunction and repressed eNOS mRNA expression were restored by the C. oil treatment. Furthermore, aortic cholesterol accumulation and CD68 expression were also reduced in

the C. oil-treated group. The effect of C. oil at 300 mg/kg was comparable with the standard drug ezetimibe. Delving into the probable anti-hyperlipidaemic mechanism at the transcript level, the C. oil-treated groups fed the chow and HC diets were compared with the chow diet-fed group. The C. oil treatment significantly increased the hepatic expression of PPARa, LXRa, CYP7A1, ABCA1, ABCG5, ABCG8 and LPL accompanied by reduced SREBP-2 and HMGCR expression. C. oil also enhanced ABCA1, ABCG5 and ABCG8 expression and suppressed NPC1L1 expression in the jejunum. In the present study, C. oil demonstrated an anti-hyperlipidaemic effect and reduced lipid-induced oxidative stress, platelet activation and vascular dysfunction. The anti-hyperlipidaemic effect exhibited by C. oil seems to be mediated by the modulation of PPARa, LXRa and associated genes involved in lipid metabolism and transport.

7) **Standardized fraction of *Xylocarpus moluccensis* inhibits inflammation by modulating MAPK-NFκB and ROS-HIF1α-PKM2 activation**.

Agarwal H, Sukka SR, Singh V, Dikshit M, Barthwal MK. **Inflamm Res.** 2022 Apr;71(4):423-437. (**I.F 6.7**)

Present study investigates the effect of Xylocarpus moluccensis (Lamk.) M. Roem fruit fraction (CDR) on endotoxemia and explores the underlying mechanisms. The effect of CDR (1-100 μg/ml) was assessed on cytokines, MAPKs, ROS, and metabolic reprogramming in LPS-induced cells (J774.2 and THP-1) by the conventional methodology of ELISA, PCR, and Western blotting. The effect of CDR (1-50 mg/kg, p.o.) was also evaluated in the mice model of endotoxemia and sepsis. CDR prevents LPS-induced cytokine production from murine and human whole blood and cell lines. CDR suppressed total cellular and mitochondrial superoxide generation and preserved mitochondrial function in LPS-stimulated phagocytes. Additionally, CDR abrogated LPS-induced MAPK's phosphorylation and IκBα degradation in J774.2 cells. Moreover, CDR suppressed LPS-induced glycolytic flux as indicated from PKM2, HK-2, PDK-2, and HIF-1α expression in J774.2 cells. In vivo, CDR pre-treatment inhibited pro-inflammatory cytokines release, metabolic reprogramming from oxidative phosphorylation to glycolysis in both LPS-induced endotoxemia and cecal slurry-induced sepsis mice model. Present study demonstrates the protective effect of CDR on LPS-induced inflammation and sepsis and identifies MAPK-NFκB and ROS-HIF1α-PKM2 as the putative target axis.

8) **Role of pyruvate kinase M2 in oxidized LDL-induced macrophage foam cell formation and inflammation.**

Kumar A, Gupta P, Rana M, Chandra T, Dikshit M, Barthwal MK. **J Lipid Res.** 2020 Mar;61(3):351-364. (**I.F: 6.5**)

Pyruvate kinase M2 (PKM2) links metabolic and inflammatory dysfunction in atherosclerotic coronary artery disease; however, its role in oxidized LDL (Ox-LDL)-induced macrophage foam cell formation and inflammation is unknown and therefore was studied. In recombinant mouse granulocyte-macrophage colony-stimulating factor-differentiated murine bone marrow-derived macrophages, early (1-6 h) Ox-LDL treatment induced PKM2 tyrosine 105 phosphorylation and promotes its nuclear localization. PKM2 regulates aerobic glycolysis and inflammation because PKM2 shRNA or Shikonin abrogated Ox-LDL-induced hypoxia-inducible factor-1α target genes lactate dehydrogenase, glucose transporter member 1, interleukin 1β (IL-1β) mRNA expression, lactate, and secretory IL-1β production. PKM2 inhibition significantly increased Ox-LDL-induced ABCA1 and ABCG1 protein expression and NBD-cholesterol efflux to apoA1 and HDL. PKM2 shRNA significantly inhibited Ox-LDL-induced CD36, FASN protein expression, DiI-Ox-LDL binding and uptake, and cellular total cholesterol, free cholesterol, and cholesteryl ester content. Therefore, PKM2 regulates lipid uptake and efflux. DASA-58, a PKM2 activator, downregulated LXR-α, ABCA1, and ABCG1, and augmented FASN and CD36 protein expression. Peritoneal macrophages showed similar results. Ox-LDL induced PKM2-SREBP-1 interaction and FASN expression in a PKM2-dependent manner. Therefore, this study suggests a role for PKM2 in Ox-LDL-induced aerobic glycolysis, inflammation, and macrophage foam cell formation.

This study suggests that targeting PKM2 can modulate atherosclerosis progression and therefore can be an attractive target for therapeutic intervention.

9) **Lin28B Regulates Angiotensin II-Mediated Let-7c/miR-99a MicroRNA Formation Consequently Affecting Macrophage Polarization and Allergic Inflammation**.

Jaiswal A, Maurya M, Maurya P, Barthwal MK. **Inflammation**. 2020 Oct;43(5):1846-1861. (**I.F. 5.1**)

Angiotensin-II (Ang-II) receptor plays a role in allergic airway inflammation; however, the underlying mechanism and role of macrophages need better understanding. In the present study, angiotensin-II infusion (1 μg/kg/min) in ovalbumin-induced airway inflammation mice model significantly decreased immune cell infiltration, goblet cell hyperplasia, and eosinophil numbers in lungs. Ang-II infusion increased M1 and decreased M2 macrophage population in bronchoalveolar lavage fluid and respective macrophage markers in lung macrophages. Similarly, in vitro Ang-II treatment in murine bone marrow-derived macrophages (BMDMs) induced M1 and reduced M2 macrophage phenotype with enhanced bactericidal activity. Mechanistically, Ang-II inhibits Let-7c and miR-99a expression in BMDMs and in vivo as well. Lentiviral overexpression of Let-7c and miR-99a miRNAs in BMDMs abrogated Ang-II-induced M1 phenotype activation and promoted M2 phenotype, which is governed by targeting TNFα by miR-99a. In lung macrophages, ovalbumin-induced TNFα inhibition was rescued after Ang-II treatment. In BMDMs, knockdown of TNFα abrogated Ang-II-induced M2 to M1 macrophage phenotype switch and associated bactericidal activity. Ang-II affects mature miRNA formation by enhancing Lin28B levels in macrophages in vivo and in vitro. Furthermore, Lin28B knockdown prevented Ang-II-mediated inhibition of mature Let-7c/miR-99a miRNA formation, M2 to M1 macrophage phenotype switch, and increased bactericidal activity. Therefore, present study suggests a role of Lin28B in Ang-II-induced Let-7c/miR-99a miRNA formation that consequently affects TNFα production, M1 phenotype activation, and allergic airway inflammation.

10) **PKCδ-IRAK1 axis regulates oxidized LDL-induced IL-1β production in monocytes.**

Tiwari RL, Singh V, Singh A, Rana M, Verma A, Kothari N, Kohli M, Bogra J, Dikshit M, Barthwal MK. **J Lipid Res.** 2014 Jul;55(7):1226-44. (**I.F. 6.5**)

This study examined the role of interleukin (IL)-1 receptor-associated kinase (IRAK) and protein kinase C (PKC) in oxidized LDL (Ox-LDL)-induced monocyte IL-1β production. In THP1 cells, Ox-LDL induced time-dependent secretory IL-1β and IRAK1 activity; IRAK4, IRAK3, and CD36 protein expression; PKCδ-JNK1 phosphorylation; and AP-1 activation. IRAK1/4 siRNA and inhibitor (INH)-attenuated Ox-LDL induced secreted IL-1β and pro-IL-1β mRNA and pro-IL-1β and mature IL-1β protein expression, respectively. Diphenyleneiodonium chloride (NADPH oxidase INH) and N-acetylcysteine (free radical scavenger) attenuated Ox-LDL-induced reactive oxygen species generation, caspase-1 activity, and pro-IL-1β and mature IL-1β expression. Ox-LDL-induced secretory IL-1β production was abrogated in the presence of JNK INH II, Tanshinone IIa, Ro-31-8220, Go6976, Rottlerin, and PKCδ siRNA. PKCδ siRNA attenuated the Ox-LDL-induced increase in IRAK1 kinase activity, JNK1 phosphorylation, and AP-1 activation. In THP1 macrophages, CD36, toll-like receptor (TLR)2, TLR4, TLR6, and PKCδ siRNA prevented Ox-LDL-induced PKCδ and IRAK1 activation and IL-1β production. Enhanced Ox-LDL and IL-1β in systemic inflammatory response syndrome (SIRS) patient plasma demonstrated positive correlation with each other and with disease severity scores. Ox-LDL-containing plasma induced PKCδ and IRAK1 phosphorylation and IL-1β production in a CD36-, TLR2-, TLR4-, and TLR6-dependent manner in primary human monocytes. Results suggest involvement of CD36, TLR2, TLR4, TLR6, and the PKCδ-IRAK1-JNK1-AP-1 axis in Ox-LDL-induced IL-1β production.

The study demonstrates the ability of oxidized LDL (Ox-LDL) in inducing sterile inflammation and the role of interlukin-1 receptor associated kinase (IRAK) in the same.

A. Patents and contribution:

1) Chiral 3-aminomethylpiperidine derivative as inhibitors of collagen induced platelet activation and adhesion.
Inventors: Dikshit DK, Dikshit MD, , Irshad T, Siddiqui, Kumar A, Bhatta R, Jain GK, Barthwal MK, Misra A, Khanna V, Prakash P, Jain M, Singh V, Gupta V & Dwivedi AK.
US Patent Granted: US9206155B2/Dec8, 2015

In this study the applicant performed the platelet aggregation studies and work in dyslipidemic hamsters.

Patent filed:
2) Pyranone fused Aza-heterocyclic fluorescent dyes useful fluorescent probes.
Inventors: Goel A, Raghuvanshi A, Jha AK, Dogra S, Yadav PN, Jaiswal A, Barthwal MK, 23/Dec/2015, 4242/DEL/2015

In this work the applicant identified the compounds specific to macrophage polarization. Compound putative targets were also identified by the applicant. The compound was synthesized by Dr Atul Goel and his group.

B. **Publications and contributions**:
3) In all the research papers mentioned above, the applicant is the corresponding author and responsible for the work.