Review Article

Exploring the Potential of Transient Receptor Potential: Troubleshooting Troublesome Calcium Thoroughfares in Biomedicine

Ammad Ahmad Farooqi[1*], Abuzar Kaleem[1], Muhammad Khalid Javeed[1], Qaisar Mansoor[2], Shahzad Bhatti[1*]

[1]*Institute of Molecular Biology and Biotechnology (IMBB)*
The University of Lahore, Pakistan
E-mail: ammadahmad638@yahoo.com, drshahzadbhatti@yahoo.com

[2]*Institute of Biomedical and Genetic Engineering (IBGE)*
Islamabad, Pakistan

[*]*Corresponding author*

Received: November 27, 2010 **Accepted: December 7, 2010**

Published: January 31 , 2011

Abstract: *Transient Receptor Potential-Canonical (TRPC) channels are the border guards residing in the supra-molecular assembly of plasma membrane. TRPCs represent a family of channels that have dual functions of store-operated and second messenger-operated channels in a diversity of cell types. Any disruption in the spatio-temporal organization drastically influences the calcium homeostasis. This review summarizes current interpretations on the infrastructure and characteristic divalent ions regulation in molecular anomalies. A specific targeting of these channels will enable us to get a step closer to personalized medicines.*

Keywords*: TRPV, TRPC, Oncogenesis, STIM, Therapeutic interventions.*

Introduction

Calcium is one of the repertoires of the cell. Calcium ions trigger multifarious processes. It implicates these processes by promoting or suppressing the interplay between proteins. These aspects range from cellular proliferation to apoptosis. Although both these parameters are paradigm in parallax, still opposite sides of the same coin. Ca^{2+} mediated signal transduction is engaged in tumorigenesis or various facets of tumor progression like metastasis, angiogenesis or invasion. Ca^{2+} is often given superficial attention or viewed simplistically as a switch. Paradoxically Ca^{2+} homeostasis must be controlled in a precise manner. There is a list of membrane associated proteins directly implicated in Ca^{2+} homeostasis and amplitude. There is no hardcore profile for variations in Ca^{2+} channel expression in cancer cells.

TRP: TR(I)P to a planet of multitasking channels

Transient receptor potential (TRP) channels behave as multifunctional cellular sensors and are engaged in appropriate switching and tuning of cellular growth factor signaling. Following literature will provide an overview of current awareness about the discrete role of TRP channels in various diseases. On the basis of phylogenetic categorization there are three major families. The TRPC or canonical TRP family, with seven members (TRPC1 through TRPC7), TRPV family comprises six members (TRPV1-6) and the TRPM family encompasses eight

members (TRPM1-8). On the basis of structural and functional commonalities, TRPC splits into four different subfamilies: TRPC1, TRPC2, TRPC3, 6 and 7 and TRPC4 and 5 (Reviewed by Montell [49]).

Increase in plasma membrane Ca^{2+} channel expression (for example in prostate cancer cells there is an up-regulation of TRPM8 and TRPV6) and reduced expression of Ca^{2+} pumps that limit the drastic increase in the ions. This malpractice would augment Ca^{2+} incline in cytosol thus facilitating proliferative signaling pathways. Significant changes in Ca^{2+} homeostasis and amplitude may take place. Ca^{2+} channel localization was altered in neoplastic cell. Expression of Ca^{2+} channel on the ER could result in Ca^{2+} leak from ER and resistance of apoptotic stimuli. In human prostate cancer cell line LNCaP, TRPM8 leaves plasma membrane and localizes to ER membrane [62]. Conversely, when overexpressed in Human Embryonic Kidney 293 Cells (HEK-293 cells), Transient receptor potential melastatin 8 (TRPM8) appeared both in plasma membrane and ER membrane.

GPCR: A versatile signalling orchestrator

Traditionally, it has been presumed that the constituents of signaling pathways show pathway fidelity and act with a high degree of autonomy. However, substantial fraction of information confirms that linear transduction cascades undergo integration. The linear pathways are often shared between multiple pathways. G protein-coupled receptors (GPCRs) respond to various ligands, such as hormones, neurotransmitters. It is quite often that GPCR-interacting partners directly mediate receptor signaling or they might act as scaffolds to operate the downstream signaling. Furthermore, GPCR-interacting proteins can have a big impact on the regulation of TRPC activities.

Agonists stimulate G-protein-coupled receptors (GPCRs 1-3), in the cell membrane (Fig. 1). GPCR1 activates the $G\alpha_s$ protein, which results in the generation of cyclic AMP (cAMP) by adenylyl cyclase (AC). Protein kinase A (PKA) is also activated which phosphorylates various substrates. $G\beta\gamma$-complex activates mitogen-activated protein kinase (MAPK) cascade, which results in the activation of extracellular signal-regulated kinase-1/2 (ERK1,2) to trigger transcriptional responses [20-22].

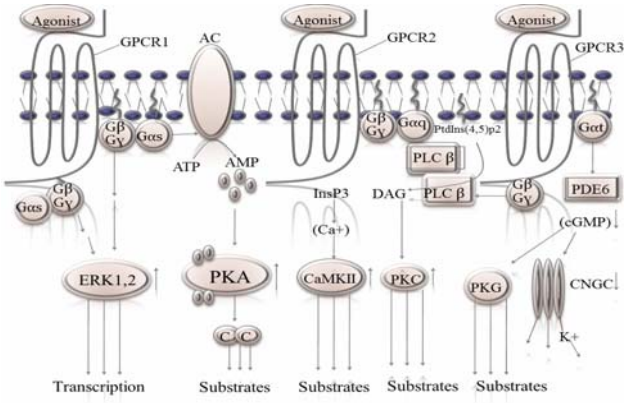

Fig. 1 Diagrammatic presentation of GPCR signaling

GPCR2 activates G protein Alpha Subunit Q polypeptide ($G\alpha_q$). Phospholipase Cβ (PLCβ), generates inositol-1,4,5-triphosphate ($InsP_3$) and diacylglycerol (DAG) from phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$). $InsP_3$ regulates intracellular Ca^{2+} ions ($[Ca^{2+}]_i$) mobilization and the activation of Ca^{2+}/calmodulin-activated protein kinase II (CaMKII) [83]. DAG activates protein kinase C (PKC) [65]. CaMKII and PKC can

phosphorylate multiple substrates [66]. GPCR3 activates G protein Alpha Subunit Transducing activity ($G\alpha_t$), which induces bipartite signaling [54], the activation of PLCβ by Gβγ-complex and the activation of phosphodiesterase-6 (PDE6). PDE6 reduces cyclic GMP, protein kinase G (PKG) activity and cyclic-nucleotide-gated channel (CNGC) activity [12, 29, 30, 64, 73].

STIM and ORAI: Dealing with TRPC in a friendly manner

A shortfall of Ca^{2+} content within the endoplasmic reticulum (ER) has to be replenished. This is triggered by receptor activation that stimulates phospholipase C (PLC) to increase inositol-1,4,5-trisphosphate ($InsP_3$) [71], leads to the opening of plasma membrane embedded Ca^{2+} release-activated Ca^{2+} (CRAC) channels. These channels are selective for Ca^{2+} and result in a cytoplasmic rise in Ca^{2+} levels. A decline in Ca^{2+} concentration below the threshold value is sensed by stromal interaction molecule 1 (STIM1), which communicates this to ORAI calcium release-activated calcium modulator (ORAI1), the pore-forming subunit of the CRAC channel (Fig. 2). STIM1 is embedded in ER membrane. Framework of the protein comprises an EF hand that binds Ca^{2+}, a "hidden" EF hand that does not bind Ca^{2+} and the sterile α-motif (SAM) domain that promotes STIM1 oligomerization. Cytoplasmic domain is equipped with functional domains, coiled-coil domain, an ezrin-radixin-moesin (ERM) domain and serine or proline-rich (S/P-rich) and lysine-rich (K-rich) segments. The CRAC activation domain (CAD) is compulsory for ORAI1 gating [1]. ORAI1 has four transmembrane domains (TM1–TM4), with intracellular amino and carboxyl termini [36-38].

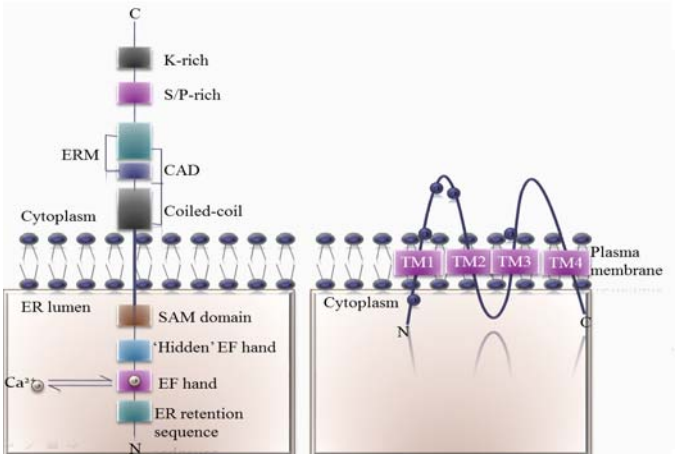

Fig. 2 Illustration of STIM and ORAI infrastructure and framework

STIM is a protein that resides in the endoplasmic reticulum [33]. It has calcium binding domains in it [18]. In the absence of calcium concentration there is a shuttling of STIM from endoplasmic reticulum to PM-ER junction [52]. It gets coupled to the Transient receptor potential canonical (TRPC) and facilitates the trafficking of calcium ions [68, 69, 75]. This phenomenon of Store operated calcium entry (SOCE) is switched on because of Ca^{2+} store depletion. There are two domains of STIM which are involved in protein aggregation/oligomerization [39, 43]. It was observed that K^{684}, K^{685} residues of STIM, that are involved in making indirect attachment with TRPC [82]. As STIM gets indirectly attached to heteromultimeried TRPC [36, 77, 81]. It first enhances heteromultimerization of TRPC isoform then gets attached to that complex. Attachment of STIM1 to TRPC dissociates pre existing interactive complex of TRPC and caveolin [50]. TRPC harbors STIM binding domain in the C terminal region of the channel while N-Terminal region holds binding site for caveolin. STIM C-terminal interacts with C-Terminal region of TRPC [23, 40].

There is a very tight involvement of ER stored calcium in pulmonary constriction. The induced mobilization of Ca^{2+} out of ER infringed the vasoconstriction. There is threshold calcium amplitude that is necessary in cytoplasm to instigate contraction. This threshold can be maintained either by ER stored depletion or influx of Ca^{2+} ions via TRPC channels. In agreement with this assumption another parameter that cannot be ruled out is that elevated cytosolic amplitude of Ca^{2+} is mediated by upstream and downstream avenues which are Ca^{2+} influx via plasma membrane localized channel and Ca^{2+} efflux via stored Ca^{2+} reservoirs in ER to sustain Ca^{2+} amplitude in cytosol. Hence it is obvious and outstanding situation in which cytosolic calcium is the licensing factor for proliferation and vasoconstriction. Both these mechanisms are bolstered by Ca^{2+} ions in cytoplasm. If this threshold amplitude of Ca^{2+} is extinguished by inhibiting Ca^{2+} mobilization via ER or abrogating influx via TRP channels, both patterns of constriction and proliferation can be attenuated [9, 28, 45, 60, 61].

TRP's are potential candidates that indicate a transitional switch from uterine quiescence to contraction during labor. Various isoforms of TRPC are localized in myometrium. Henceforth similar contractions are induced by increase in cytosolic Ca^{2+} ions which facilitate the process [16, 17]. Pulmonary vasoconstriction is a phenomenon associated with constriction of pulmonary arteries in hypoxia (low oxygen level) without hypercapnia (high carbon dioxide level). This hypoxic stress redirects blood flow to higher oxygen content region i.e is alveoli. Vasoconstriction is more pronounced in distal pulmonary artery than proximal artery. This vasoconstriction is triggered by co-interaction of TRPC and STIM-C that impinges store operated calcium entry [41, 42]. IP3 mediated Icat (IP3 induced nonselective cation current) activation and vasoconstriction by IP3R1 and TRPC3 present in arterial myocytes [72]. C terminal calmodulin and IP3R binding (CIRB) domain are present in myocyte TRPC3 and TRPC6 channels [2, 8, 25, 70] determined the role of TRPC in pulmonary vasoconstriction. They used epoxyeicosatrienoic acid (EET) in TRPC heterozygous and homozygous mice to explore the involvement of TRPC6 in pulmonary vasoconstriction. There was a remarkable vasoconstriction in TRPC heterozygous mice but contrarily, there was no effect of compound EET on the mice, that were homozygous recessive for TRPC. These observations strengthen our understanding regarding role of TRPC in pulmonary vasoconstriction. TRPC is activated by G-protein coupled receptor signaling in an indirect manner. PLC once switched on because of GPCR signaling acts on Phosphatidylinositolbisphosphate (PIP2) and subsequently generation of IP3 and DAG takes place [32, 34]. Both entities are implicated in activation of TRPC [46]. TRPC4 expression is upregulated in diabetic vessels whereas TRPC1 and TRPC6 are downregulated [14].

Heteromultimer TRPC6/TRPC7 is strongly influenced by extracellular Ca^{2+} concentration. Increase in Ca^{2+} concentration attenuates heteromultimer mediated current. Same trend was observed after knockout of STIM which compromised trafficking of Ca^{2+} ions after store depletion. STIM ablation prevented metastatic dissemination but bolstered proliferation [44].

BDNF and TRPC: Hands in glove

The binding of brain derived neurotrophic factor (BDNF) to Tropomyosin-Related Kinase B (TrkB) activates phospholipase Cγ (PLC-γ) which generates diacylglycerol (DAG) and inositol triphosphate (IP_3) [5, 15]. DAG directly activates TRP family channels [3, 35, 59], whereas IP_3 may trigger store-operated Ca^{2+} release mechanisms [63, 76, 79]. Ca^{2+} influx via TRPC3 and TRPC6 channels activates extracellular receptor kinase (Erk) and Calcium/calmodulin-dependent protein kinase type IV (CaMKIV). This activates cAMP responsive element binding (CREB) transcriptional pathways that integrate with

Akt-dependent pathways to potentiate survival of neurons. Ca^{2+}-dependent survival pathways are induced via activity-dependent opening of L-type Ca^{2+} channels as well (Fig. 3).

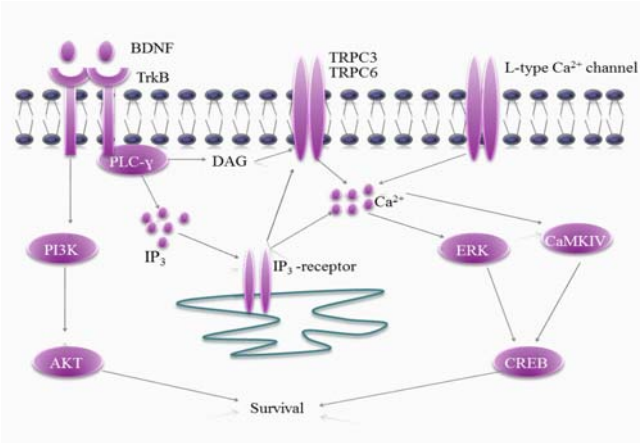

Fig. 3 BDNF-induced and activity-dependent survival mechanisms are potentiated by intricate Ca^{2+}-dependent signaling pathways

Activation cascades of TRPC

TRPC is susceptible to stimulation by multiple factors. Sustainability of TRPC6 in plasma membrane is bolstered by Ras-like proteins (Rab 9 and Rab 11). Both the proteins are actively involved in recycling of TRPC6 to plasma membrane (Fig. 4) [11]. Substance P (subp) and IP3 are implicated in activating TRPC's. IP3 displaces PIP2 mediated inhibition of TRPC7 subunits [6, 24].

SEC14 domain and spectrin repeat-containing protein 1 (SESTD1) gets docked to calmodulin and IP3 receptor domain in TRP-like protein (TRPL). SESTD1 harbors a lipid binding SEC14 domain. Multiple phospholipids get attached to SESTD1 which render it active and enhance its affinity for TRPC [47]. TRPC mediated inward trafficking of calcium ions facilitates the expression of vascular cell adhesion molecule (VCAM) via tumor necrosis factor (TNF) and Adenosine triphosphate (ATP) [57, 58]. Notch signaling modulates transcription of TRPC via NUMB [13, 31].

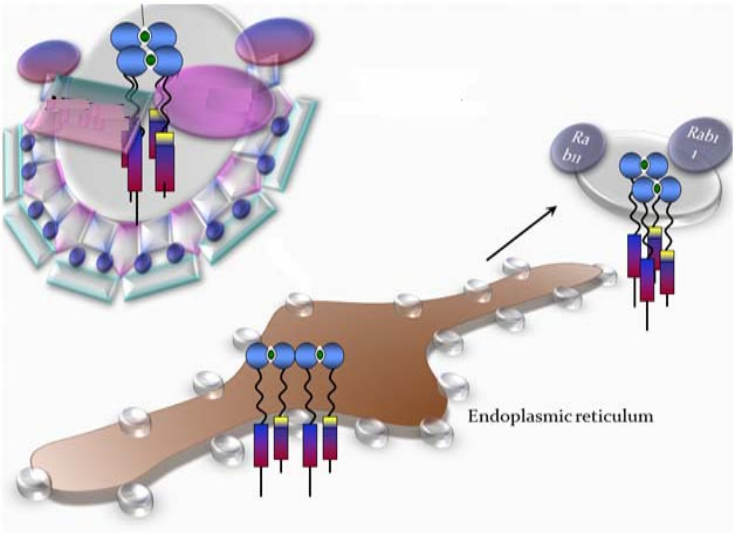

Fig. 4 Tight regulation of TRPC by Rab proteins

TRPC: Partners and partnerships in the crime

Facts advocate that up-regulation of TRPC channels is entailed in the development of cardiac hypertrophy and heart failure. Moreover it contributes in vascular remodeling and pulmonary hypertension. TRP channels are imperative novel pharmacological targets for the clinical management of cardiovascular disease.

Atrial natriuretic peptides (ANP) and brain natriuretic peptides (BNP) are actively involved in mediating antihypertrophic effects in the heart via their common receptor, guanylyl cyclase (GC)-A. It generates cGMP, for activation of protein kinase (PK)G. Protein kinaseG (PKG) suppresses TRPC6 activity and cardiac hypertrophy. It was unclear whether PKG suppresses cardiac hypertrophy through inhibition of TRPC. PKG activated by Phosphodiesterase5 (PDE5) inhibition phosphorylated TRPC6 proteins at Thr(69). This interferes with the calcium trafficking. Substitution of Ala for Thr(69) in TRPC6 impaired the anti-hypertrophic effects of PDE5 inhibition. PDE5 inhibitiors induce TRPC6 phosphorylation that plays an active role in prevention of pathological hypertrophy by PDE5 inhibition. Overexpressed TRPC6 in GC-A compromised cells exacerbated cardiac hypertrophy as TRPC channel blocker, considerably decreased the cardiac hypertrophy. There is an existence of an interactive pathway encompassing TRPC activation via cardiac ANP/BNP-GC-A pathway. TRPC6 blockade is an effective therapeutic strategy for management of pathological cardiac remodeling [26, 27, 51].

In cardiomyocytes, alterations in the frequency or amplitude of calcium results in induction of hypertrophy. Some pivotal receptor-ligand engagements regulate the switching of calcium channels in a diversified manner. Angiotensin (Ang) II modulates pathogenesis of cardiac hypertrophy by nuclear factor of activated T cells (NFAT), a calcium responsive transcriptional factor. PLC-mediated production of diacylglycerol (DAG) results in robust activation of diacylglycerol (DAG)-responsive canonical transient receptor potential (TRPC) subfamily channels (TRPC3 and TRPC6) triggers membrane depolarization. Ablation of either TRPC3 or TRPC6 completely hampered agonist-induced hypertrophic responses, highlighting that TRPC3 and TRPC6 undergo heterotetramerization and targeting of either member of the family disrupts the synergy.

A recent swing of emphasis towards endogenous TRPC proteins has resulted in a mounting body of substantiation indicating that a combinatorial scheme is observed in TRPC channels. These proteins work synchronously to form native heteromeric SOCs. TRPC proteins are involved in the formation of multiple native channel types. Heteromultimerization of variety of TRPC proteins, results in assembly into multifarious channel types.

Interestingly there is an antagonistic interaction that prevails between TRPC3/TRPC6 and TRPC1/TRPC5. TRPC6 channels evoked by Ang II are rendered functionally inactive by TRPC1/C5-mediated Ca^{2+} influx and stimulation of PKC which phosphorylates TRPC6 subunits. This unmasks a novel interaction between two distinctive vasoconstrictor-activated TRPC channels expressed in the same VSMCs. The endothelin-1 (ET-1) receptor subtypes have a dual mode of activation of TRPC's. The alternative pathways of PIP2 and PIP3 are utilized in a precise manner to trigger the activation of the channels. This appears to be a "double edged sword" as any medication that solely targets any of the members will be incompetent to produce desired results because of the probabilistic switching mode of the signaling. Mechanistic activation of TRPC1/C5/C6 channels (termed TRPC1 channels) by stimulation of (ET-1) receptor subtypes is intriguingly diverse. Activation of native

TRPC1/C5/C6 channels executed by endothelin-1 can be achieved by both PIP3 and PIP2 [53, 55].

In pulmonary arterial smooth muscle cells (PASMCs), Ca^{2+} influx occurs primarily through store-operated Ca^{2+} channels. Exposure to prolonged hypoxia results in an upregulated TRPC1 and TRPC6 expression in PASMCs. Sildenafil, a type V phosphodiesterase inhibitor is a remarkable agent for treatment of pulmonary hypertension that elevates cellular cGMP. It alters basal calcium level in PASMCs by decrease in SOCE through TRPC1/TRPC6 expression inhibition and decreasing the vascular tone of pulmonary arteries [41, 67].

Many receptor tyrosine kinases potentiate the activities of TRPC. A "transactivation" mechanism, which links these events in one signalling chain. Platelet drived growth factor (PDGF) mediated pulmonary artery smooth muscle cell proliferation is associated with upregulation of TRPC6 expression. Increase in capacitative Ca^{2+} entry (CCE) facilitate return of Ca^{2+} to sarcoplasmic reticulum and promote pulmonary artery smooth muscle cell (PASMC) growth [74]. Intracellular Ca^{+} depletion activates capacitative Ca^{2+} entry (CCE) which takes part in Pulmonary artery (PA) concentration through TRP Ca^{2+} channels [7, 45]. Arg8-vasopressin (AVP) acts as a ligand and gets attached to vasopressin receptor present on the surface of the cell. As soon as ligand receptor engagement takes place, there is a medley of receptors which synchronize and orchestrate calcium trafficking. TRPC might work in collaboration with Platelet-drived growth factor receptor (PDGFR), because the G-protein mediated calcium mobilization was observed in case of Platelet-drived growth factor receptor (PDGFR) activation. It had very tight interaction with TRPC that acts as a channel for calcium trafficking [48]. JAK-STAT pathway induced the expression of TRPC. STAT3 moves in the nucleus, it gets attached to response elements specified for binding of STAT3. This attachment induces expression of TRPC, however if TRPC is abolished, the trafficking of calcium through this channel will be impaired despite the activation of vasopressin receptor or PDGFR.

Patients suffering from glioblastoma multiforme, the most aggressive form of glioma, have a median survival of around 12 months. Some members of the $Ca^{(2+)}$-permeable transient receptor potential canonical (TRPC) family of channel proteins are instrumental to uncontrolled division of cells. TRPC6 might be a new target for therapeutic intervention of human glioma as manipulating the pathways augmenting the dynamics of these proteins can help a lot in standardization of therapy [19].

Role of TRP's in prostate cancer progression

Prostate cancer is a multifactorial disease. A wide range of proteins have been characterized and incriminated to be involved in the disease progression. A confluence of observations indicates that super-families of transient receptor potential (TRPs) channel are instrumental in prostate carcinogenesis. TRPC6 [78] is associated with the disease exacerbation and knocking down of TRPC6 (69) and TRPV6 [80] was growth inhibitory in prostate cancer cell lines. Recently a research group documented a tight association of TRPV with ATM. They registered that activation of TRPV1 with capsaicin resulted in the activation of ATM alongwith induction of apoptosis [4]. A closer look at the credentials of ATM indicates that it has bipartite activities. Either it is involved in the induction of apoptosis or it is engaged in DNA repair in a faithful manner. Despite the growing evidence that these channels and genomic rearrangements have wide contributions in disease aggressiveness, no study to date addresses the tight association of the abrogation of channels and genomic rearrangements. In a set of experimentations we have evaluated influence of TRP channels in chromosomal

fusions. Silencing of these channels alongwith erlotinib resulted in activation of ATM which triggered faithful recapitulation of genome (Ammad Farooqi unpublished observations).

Gastric cancers: Opportunities and existing challenges

TRPC6 has an imperative role in human gastric cancer development. While drawing a parallel between gastric cancer cells and normal gastric epithelial cells, expression of TRPC is enhanced in former. Drug treatment of human gastric cancer cell lines, with SKF96365, an agent known to inhibit TRPC channels, detained cell cycle and cell growth [10]. Oesophageal squamous cell carcinoma (OSCC) is one of the foremost causes of cancer-related death worldwide. TRPC6 is robustly expressed in OSCC and is essential for cell proliferation and cell cycle. Inhibition of TRPC6 channels in human OSCC cells brings forth anti proliferative effects and induces G2/M phase arrest [56].

Conclusion and future directions

The next decade of research on TRPC channels should focus on several issues. First, it is essential to bridge the existing gaps in the literature that limit understanding of the modes of regulation of these channels. Interpreting and evaluating the range of effects of expression and factors that potentiate or blunt the expression can help to streamline these inconsistencies. As a final point, there is a need to move towards investigation of their molecular insights of cells in actual physiological systems. To this end, requirement to rely on a greater extent on knock-down strategies such as knock-out animals and RNAi gene silencing cannot be overlooked. Optimistically in the near future a sharper picture of the mechanistic insights for TRPC channels will be achieved.

References

1. Abdullaev I. F., J. Bisaillon, M. Potier, J. Gonzalez, R. Motiani, M. Trebak (2008). STIM1 and ORAI1 mediate CRAC currents and store-operated calcium entry important for endothelial cell proliferation, Circ Res, 103, 1289-1299.
2. Adebiyi A., G. Zhao, D. Narayanan, C. Thomas, J. Bannister, J. Jaggar (2010). Isoform-selective physical coupling of TRPC3 channels to IP3 receptors in smooth muscle cells regulates arterial contractility, Circulation research, 106, 1603-1612.
3. Aires V., A. Hichami, G. Boulay, N. Khan (2007). Activation of TRPC6 calcium channels by diacylglycerol (DAG)-containing arachidonic acid: a comparative study with DAG-containing docosahexaenoic acid, Biochimie, 89, 926-937.
4. Amantini C., P. Ballarini, S. Caprodossi, M. Nabissi, M. Morelli, R. Lucciarini, M. Cardarelli, G. Mammana, G. Santoni (2009). Triggering of transient receptor potential vanilloid type 1 (TRPV1) by capsaicin induces Fas/CD95-mediated apoptosis of urothelial cancer cells in an ATM-dependent manner, Carcinogenesis, 8, 1320-1329.
5. Amaral M. D., L. Pozzo-Miller (2007). BDNF induces calcium elevations associated with IBDNF, a nonselective cationic current mediated by TRPC channels, J Neurophysiol, 98, 2476-2482.
6. Ben-Mabrouk, F., A. Tryba (2010). Substance P modulation of TRPC3/7 channels improves rhythm regularity and ICAN-dependent pacemaker activity, Eur J Neurosci, 31, 1219-1232.
7. Birnbaumer L., G. Boulay, D. Brown, M. Jiang, A. Dietrich, K. Mikoshiba, X. Zhu, N. Qin (2000). Mechanism of capacitative Ca^{2+} entry (CCE): interaction between IP3 receptor and TRP links the internal calcium storage compartment to plasma membrane CCE channels, Recent Prog Horm Res, 55, 127-161.

8. Boulay G., D. Brown, N. Qin, M. Jiang, A. Dietrich, M. Zhu, Z. Chen, M. Birnbaumer, K. Mikoshiba, L. Birnbaumer (1999). Modulation of $Ca^{(2+)}$ entry by polypeptides of the inositol 1,4,5-trisphosphate receptor (IP3R) that bind transient receptor potential (TRP): evidence for roles of TRP and IP3R in store depletion-activated $Ca^{(2+)}$ entry, Proc Natl Acad Sci USA, 96, 14955-14960.
9. Brueggemann L. I., D. Markun, K. Henderson, L. Cribbs, K. Byron (2006). Pharmacological and electrophysiological characterization of store-operated currents and capacitative $Ca^{(2+)}$ entry in vascular smooth muscle cells, J Pharmacol Exp Ther, 317, 488-499.
10. Cai R., X. Ding, K. Zhou, Y. Shi, R. Ge, G. Ren, Y. Jin, Y. Wang (2009). Blockade of TRPC6 channels induced G2/M phase arrest and suppressed growth in human gastric cancer cells, Int J Cancer, 125, 2281-2287.
11. Cayouette S., S. Bousquet, N. Francoeur, E. Dupré, M. Monet, H. Gagnon, Y. Guedri, C. Lavoie, G. Boulay (2010). Involvement of Rab9 and Rab11 in the intracellular trafficking of TRPC6, Biochim Biophys Acta, 1803, 805-812.
12. Chen J., R. Crossland, M. Noorani, S. Marrelli (2009). Inhibition of TRPC1/TRPC3 by PKG contributes to NO-mediated vasorelaxation, Am J Physiol Heart Circ Physiol, 297, H417-H424.
13. Chigurupati S., R. Venkataraman, D. Barrera, A. Naganathan, M. Madan, L. Paul, J. Pattisapu, G. Kyriazis, K. Sugaya, S. Bushnev, J. Lathia, J. Rich, S. Chan (2010). Receptor channel TRPC6 is a key mediator of notch-driven glioblastomagrowyh and invasiveness, Cancer Res, 70, 418-427.
14. Chung A. W., A. Yeung, E. Chum, E. Okon, C. van Breemen (2009). Diabetes modulates capacitative calcium entry and expression of transient receptor potential canonical channels in human saphenous vein, Eur J Pharmacol, 613, 114-118.
15. Ciobanu C., G. Reid, A. Babes (2009). Acute and chronic effects of neurotrophic factors BDNF and GDNF on responses mediated by thermo-sensitive TRP channels in cultured rat dorsal root ganglion neurons, Brain Res, 1284, 54-67.
16. Dalrymple A., D. Slater, D. Beech, L. Poston, R. Tribe (2002). Molecular identification and localization of Trp homologues, putative calcium channels, in pregnant human uterus, Mol Hum Reprod, 8, 946-951.
17. Dalrymple A., D. Slater, D. Beech, L. Poston, R. Tribe (2004). Physiological induction of transient receptor potential canonical proteins, calcium entry channels, in human myometrium: influence of pregnancy, labor, and interleukin-1 beta, J Clin Endocrinol Metab, 89, 1291-1300.
18. Darbellay B., S. Arnaudeau, D. Ceroni, C. Bader, S. Konig, L. Bernheim (2010). Human muscle economy: myoblast differentiation and excitation/contraction coupling use the same molecular partners, STIM1 and STIM2, J Biol Chem, 285, 22437-22447.
19. Ding X., Z. He, K. Zhou, J. Cheng, H. Yao, D. Lu, R. Cai, Y. Jin, B. Dong, Y. Xu, Y. Wang (2010). Essential role of TRPC6 channels in G2/M phase transition and development of human glioma, J Natl Cancer Inst, 102, 1052-1068.
20. El Hiani Y., A. Ahidouch, V. Lehen'kyi, F. Hague, F. Gouilleux, R. Mentaverri, S. Kamel, K. Lassoued, G. Brûlé, H. Ouadid-Ahidouch (2009). Extracellular signal-regulated kinases 1 and 2 and TRPC1 channels are required for calcium-sensing receptor-stimulated MCF-7 breast cancer cell proliferation, Cell Physiol Biochem, 23, 335-346.
21. El Hiani Y., A. Ahidouch, M. Roudbaraki, S. Guenin, G. Brûlé, H. Ouadid-Ahidouch (2006). Calcium-sensing receptor stimulation induces nonselective cation channel activation in breast cancer cells, J Membr Biol, 211, 127-137.

22. Goel M., C. Zuo, W. Schilling (2010). Role of cAMP/PKA signaling cascade in vasopressin-induced trafficking of TRPC3 channels in principal cells of the collecting duct, Am J Physiol Renal Physiol, 298, F988-F996.
23. Huang G. N. et al. (2006). STIM1 carboxyl-terminus activates native SOC, I (crac) and TRPC1 channels, Nat Cell Biol, 8, 1003-1010.
24. Ju M., J. Shi, S. Saleh, A. Albert, W. Large (2010). Ins (1,4,5)P3 interacts with PIP2 to regulate activation of TRPC6/C7 channels by diacylglycerol in native vascular myocytes, J Physiol, 588, 1419-1433.
25. Keserü B., E. Barbosa-Sicard, R. Popp, B. Fisslthaler, A. Dietrich, T. Gudermann, B. Hammock, J. Falck, N. Weissmann, R. Busse, I. Fleming (2008). Epoxyeicosatrienoic acids and the soluble epoxide hydrolase are determinants of pulmonary artery pressure and the acute hypoxic pulmonary vasoconstrictor response, FASEB J, 22, 4306-4315.
26. Kinoshita H., K. Kuwahara, M. Nishida, Z. Jian, X. Rong, S. Kiyonaka, Y. Kuwabara, H. Kurose (2010). Inhibition of TRPC6 channel activity contributes to the antihypertrophic effects of natriuretic peptides – guanylyl cyclase – a signaling in the heart, Circ Res, 106, 1849-1860.
27. Koitabashi N., T. Aiba, G. Hesketh, J. Rowell, M. Zhang, E. Takimoto, G. Tomaselli, D. Kass (2010). Cyclic GMP/PKG-dependent inhibition of TRPC6 channel activity and expression negatively regulates cardiomyocyte NFAT activation novel mechanism of cardiac stress modulation by PDE5 inhibition, J Mol Cell Cardiol, 48, 713-724.
28. Kunichika N., Y. Yu, C. Remillard, O. Platoshyn, S. Zhang, J. Yuan (2004). Overexpression of TRPC1 enhances pulmonary vasoconstriction induced by capacitative Ca^{2+} entry, Am J Physiol Lung Cell Mol Physiol, 287, 962-969.
29. Kwan H. Y., Y. Huang, X. Yao (2004). Regulation of canonical transient receptor potential isoform 3 (TRPC3) channel by protein kinase G, Proc Natl Acad Sci USA, 101, 2625-2630.
30. Kwan H. Y., Y. Huang, X. Yao (2006). Protein kinase C can inhibit TRPC3 channels indirectly via stimulating protein kinase G, J Cell Physiol, 207, 315-321.
31. Kyriazis G. A., C. Belal, M. Madan, D. G. Taylor, J. Wang, Z. Wei, J. V. Pattisapu, S. L. Chan (2010). Stress-induced switch in Numb isoforms enhances Notch-dependent expression of subtype-specific transient receptor potential channel, J Biol Chem, 285, 6811-6825.
32. Large W. A., S. N. Saleh, A. P. Albert (2009). Role of phosphoinositol 4,5-bisphosphate and diacylglycerol in regulating native TRPC channel proteins in vascular smooth muscle, Cell Calcium, 45, 574-582.
33. Lee K. P., J. P. Yuan, J. H. Hong, I. So, P. F. Worley, S. Muallem (2010). An endoplasmic reticulum/plasma membrane junction: STIM1/ORAI1/TRPCs, FEBS Lett, 584, 2022-2027.
34. Leung H. T., J. Tseng-Crank, E. Kim, C. Mahapatra, S. Shino, Y. Zhou, L. An, R. W. Doerge, W. L. Pak (2008). DAG lipase activity is necessary for TRP channel regulation in Drosophila photoreceptors, Neuron, 58, 884-896.
35. Li H. S., X. Z. Xu, C. Montell (1999). Activation of a TRPC3-dependent cation current through the neurotrophin BDNF, Neuron, 24, 261-273.
36. Liao Y., C. Erxleben, J. Abramowitz, V. Flockerzi, M. X. Zhu, D. L. Armstrong, L. Birnbaumer (2008). Functional interactions among ORAI1, TRPCs, and STIM1 suggest a STIM-regulated heteromeric ORAI/TRPC model for SOCE/Icrac channels, Proc Natl Acad Sci USA, 105, 2895-2900.
37. Liao Y., C. Erxleben, E. Yildirim, J. Abramowitz, D. L. Armstrong, L. Birnbaumer (2007). ORAI proteins interact with TRPC channels and confer responsiveness to store depletion, Proc Natl Acad Sci USA, 104, 4682-4687.

38. Liao Y., N. W. Plummer, M. D. George, J. Abramowitz, M. X. Zhu, L. Birnbaumer (2009). A role for ORAI in TRPC-mediated Ca^{2+} entry suggests that a TRPC: ORAI complex may mediate store and receptor operated Ca^{2+} entry, Proc Natl Acad Sci USA, 106, 32026.
39. Liou J., M. Fivaz, T. Inoue, T. Meyer (2007). Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca^{2+} store depletion, Proc Natl Acad Sci USA, 104, 9301-9306.
40. Lockwich T. P. et al. (2000). Assembly of Trp1 in a signaling complex associated with caveolin-scaffolding lipid raft domains, J Biol Chem, 275, 11934-11942.
41. Lu W., P. Ran, D. Zhang, G. Peng, B. Li, N. Zhong, J. Wang (2010). Sildenafil inhibits chronically hypoxic upregulation of canonical transient receptor potential expression in rat pulmonary arterial smooth muscle, Am J Physiol Cell Physiol, 298, 114-123.
42. Lu W., J. Wang, L. A. Shimoda, J. T. Sylvester (2008). Differences in STIM1 and TRPC expression in proximal and distal pulmonary arterial smooth muscle are associated with differences in Ca^{2+} responses to hypoxia, Am J Physiol Lung Cell Mol Physiol, 295, L104-L113.
43. Luik R. M., B. Wang, M. Prakriya, M. M. Wu, R. S. Lewis (2008). Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation, Nature, 454, 538-542.
44. Maruyama Y., Y. Nakanishi, E. J. Walsh, D. P. Wilson, D. G. Welsh, W. C. Cole (2006). Heteromultimeric TRPC6-TRPC7 channels contribute to arginine vasopressin-induced cation current of A7r5 vascular smooth muscle cells, Circulation Research, 98, 1520.
45. McDaniel S. S., O. Platoshyn, J. Wang, Y. Yu, M. Sweeney, S. Krick, L. I. Rubin, J. X. Yuan (2001). Capacitative $Ca^{(2+)}$ entry in agonist-induced pulmonary vasoconstriction, Am J Physiol Lung Cell Mol Physiol, 280, L870-L880.
46. Mederos Y., M. Schnitzler, U. Storch, S. Meibers, P. Nurwakagari, A. Breit, K. Essin, M. Gollasch, T. Gudermann (2008). Gq-coupled receptors as mechanosensors mediating myogenic vasoconstriction, EMBO J, 27, 3092-3103.
47. Miehe S., A. Bieberstein, I. Arnould, O. Ihdene, H. Rütten, C. Strübing (2010). The phospholipid-binding Protein SESTD1 Is a novel regulator of the transient receptor potential channels TRPC4 and TRPC5, J Biol Chem, doi M109, 068304.
48. Moneer Z., I. Pino, E. J. Taylor, L. M. Broad, Y. Liu, S. C. Tovey, L. Staali, C. W. Taylor (2005). Different phospholipase-C-coupled receptors differentially regulate capacitative and non-capacitative Ca^{2+} entry in A7r5 cells, Biochem J, 389, 821-829.
49. Montell C. (2005). The TRP superfamily of cation channels, Sci STKE, 272.
50. Ng L. C. et al. (2009). TRPC1 and STIM1 mediate capacitative Ca^{2+} entry in mouse pulmonary arterial smooth muscle cells, J Physiol, doi 10.1113/jphysiol.172254.
51. Nishida M., K. Watanabe, Y. Sato, M. Nakaya, N. Kitajima, T. Ide, R. Inoue, H. Kurose (2010). Phosphorylation of TRPC6 channels at Thr69 is required for anti-hypertrophic effects of phosphodiesterase 5 inhibition, J Biol Chem, 285, 13244-13253.
52. Roos J., et al. (2005). STIM1, an essential and conserved component of store-operated Ca^{2+} channel function, J Cell Biol, 169, 435-445.
53. Saleh S. N., A. P. Albert, W. A. Large (2009). Activation of native TRPC1/C5/C6 channels by endothelin-1 is mediated by both PIP3 and PIP2 in rabbit coronary artery myocytes, J Physiol, 587, 5361-5375.
54. Saleh S. N., A. P. Albert, C. M. Peppiatt-Wildman, W. A. Large (2008). Diverse properties of store-operated TRPC channels activated by protein kinase C in vascular myocytes, J Physiol, 586, 2463-2476.
55. Shi J., M. Ju, S. N. Saleh, A. P. Albert, W. A. Large (2010). TRPC6 channels stimulated by angiotensin II are inhibited by TRPC1/C5 channel activity through a Ca^{2+}- and PKC-dependent mechanism in native vascular myocytes, J Physiol, 588(19), 3671-3682.

56. Shi Y., X. Ding, Z. H. He, K. C. Zhou, Q. Wang, Y. Z. Wang (2009). Critical role of TRPC6 channels in G2 phase transition and the development of human oesophageal cancer, Gut, 58, 1443-1450.
57. Smedlund K., J. Y. Tano, G. Vazquez (2010). The constitutive function of native TRPC3 channels modulates vascular cell adhesion molecule-1 expression in coronary endothelial cells through nuclear factor {Kappa} B signaling, Circ Res, 106, 1479.
58. Smedlund K., G. Vazquez (2008). Involvement of native TRPC3 proteins in ATP-dependent expression of VCAM-1 and monocyte adherence in coronary artery andothelial cells, Arterioscler Thromb Vasc Biol, 28, 2049-2055.
59. Sossin W. S., P. A. Barker (2007). Something old, something new: BDNF-induced neuron survival requires TRPC channel function, Nat Neurosci, 10, 537-538.
60. Sweeney M, S. McDaniel, O. Platoshyn, S. Zhang, Y. Yu, B. R. Lapp, Y. Zhao, P. A. Thistlethwaite, J. X. Yuan (2002). Role of capacitative Ca^{2+} entry in bronchial contraction and remodeling, J Appl Physiol, 92, 1594-1602.
61. Sweeney M., Y. Yu, O. Platoshyn, S. Zhang, S. S. McDaniel, J. X. Yuan (2002). Inhibition of endogenous TRP1 decreases capacitative Ca^{2+} entry and attenuates pulmonary artery smooth muscle cell proliferation, Am J Physiol Lung Cell Mol Physiol, 283, 144-155.
62. Sydorenko V., Y. Shuba , S. Thebault , M. Roudbaraki, G. Lepage, N. Prevarskaya, R. Skryma (2003). Receptor-coupled, DAG-gated Ca^{2+}-permeable cationic channels in LNCaP human prostate cancer epithelial cells, J Physiol, 548, 823-836.
63. Tai K., M. C. Hamaide, H. Debaix, P. Gailly, M. Wibo, N. Morel (2008). Agonist-evoked calcium entry in vascular smooth muscle cells requires IP3 receptor-mediated activation of TRPC1, Eur J Pharmacol, 583, 135-147.
64. Takahashi S., H. Lin, N. Geshi, Y. Mori, Y. Kawarabayashi, N. Takami, M. X. Mori, A. Honda, R. Inoue (2008). Nitric oxide-cGMP-protein kinase G pathway negatively regulates vascular transient receptor potential channel TRPC6, J Physiol, 586, 4209-4223.
65. Venkatachalam K., F. Zheng, D. L. Gill (2003). Regulation of canonical transient receptor potential (TRPC) channel function by diacylglycerol and protein kinase C, J Biol Chem, 278, 29031-29040.
66. Venkatachalam K., F. Zheng, D. L. Gill (2004). Control of TRPC and store-operated channels by protein kinase C, Novartis Found Symp, 258, 172-185.
67. Wang C., J. Wang , L. Zhao, Y. Wang, J. Liu, L. Shi , M. Xu, C. Wang (2008). Sildenafil inhibits human pulmonary artery smooth muscle cell proliferation by decreasing capacitative Ca^{2+} entry, J Pharmacol Sci, 108, 71-78.
68. Wang Y., X. Deng, T. Hewavitharana, J. Soboloff, D. L. Gill (2008). STIM, ORAI and TRPC channels in the control of calcium entry signals in smooth muscle, Clin Exp Pharmacol Physiol, 35, 1127-1133.
69. Wang Y., D. Yue, K. Li, Y. Liu, C. Ren, P. Wang (2010). The role of TRPC6 in HGF-induced cell proliferation of human prostate cancer DU145 and PC3 cells, Asian J Androl, 12(6), 841-852.
70. Wedel B. J., G. Vazquez, R. R. McKay, G. St. J. Bird, J. W. J. R. Putney (2003). A calmodulin/inositol 1,4,5-trisphosphate (IP3) receptor-binding region targets TRPC3 to the plasma membrane in a calmodulin/IP3 receptor-independent process, J Biol Chem, 278, 25758-25765.
71. Woodard G. E., J. J. López, I. Jardín, G. M. Salido, J. A. Rosado (2010). TRPC3 regulates agonist-stimulated Ca^{2+} mobilization by mediating the interaction between type I inositol 1,4,5-trisphosphate receptor, RACK1 and ORAI1, J Biol Chem, 285, 8045-8053.

72. Xi Q., A. Adebiyi, G. Zhao, K. E. Chapman, C. M. Waters, A. Hassid, J. H. Jaggar (2008). IP3 constricts cerebral arteries via IP3 receptor-mediated TRPC3 channel activation and independently of sarcoplasmic reticulum Ca^{2+} release, Circ Res, 102, 1118-1126.
73. Yao X. (2007). TRPC, cGMP-dependent protein kinases and cytosolic Ca^{2+}, Handb Exp Pharmacol, 179, 527-540.
74. Yu Y., M. Sweeney, S. Zhang, O. Platoshyn, J. Landsberg, A. Rothman, J. X. Yuan (2003). PDGF stimulates pulmonary vascular smooth muscle cell proliferation by upregulating TRPC6 expression, Am J Physiol Cell Physiol, 284, C316-C330.
75. Yuan J. P., M. S. Kim, W. Zeng, D. M. Shin, G. Huang, P. F. Worley, S. Muallem (2009). TRPC channels as STIM1-regulated SOCs, Channels (Austin), 3, 221-225.
76. Yuan J. P., K. Kiselyov, D. M. Shin, J. Chen, N. Shcheynikov, S. H. Kang, M. H. Dehoff, M. K. Schwarz, P. H. Seeburg, S. Muallem, P. F. Worley (2003). Homer binds TRPC family channels and is required for gating of TRPC1 by IP3 receptors, Cell, 114, 777-789.
77. Yuan J. P., W. Zeng, G. N. Huang, P. F. Worley, S. Muallem (2007). STIM1 heteromultimerizes TRPC channels to determine their function as store-operated channels, Nat Cell Biol, 9, 636-645.
78. Yue D., Y. Wang, J. Xiao, P. Wang, C. Ren (2009). Expression of TRPC6 in benign and malignant human prostate tissues, Asian J Androl, 11, 541-547.
79. Zarayskiy V., F. Monje, K. Peter, P. Csutora, B. I. Khodorov, V. M. Bolotina (2007). Store-operated ORAI1 and IP3 receptor-operated TRPC1 channel, Channels (Austin), 1, 246-252.
80. Zhao X. Z., H. Guo, G. Liu, C. Ji, S. Zhang, T. Liu, W. Gan, X. Li (2010). Inhibitory effect of TRPV6 silencing on prostate cancer cell line LNCaP in vitro, Zhonghua Nan Ke Xue, 16, 423-427.
81. Zeng W., et al. (2008). STIM1 gates TRPC channels, but not ORAI1, by electrostatic interaction, Mol Cell, 32, 439-448.
82. Zhang S. L., Y. Yu, J. Roos, J. A. Kozak, T. J. Deerinck, M. H. Ellisman, K. A. Stauderman, M. D. Cahalan (2005). STIM1 is a Ca^{2+} sensor that activates CRAC channels and migrates from the Ca^{2+} store to the plasma membrane, Nature, 437, 902-905.
83. Zhu M. X., J. Tang (2004). TRPC channel interactions with calmodulin and IP3 receptors, Novartis Found Symp, 25, 44-58.

Assist. Prof. Ammad Ahmad Farooqi, M.Phil., M.Sc.
E-mail: ammadahmad638@yahoo.com

Ammad Ahmad Farooqi is a graduate from Government College University, Lahore. He has done his M.Sc. and M.Phil. from Quaid-i-Azam University, Islamabad. Since 2008 he is an assistant professor in the Molecular Biology Department of the University of Lahore and Member of World Academy of Science, Engineering and Technology (WASET). He is the group leader of Prostate cancer therapy group. Currently he is working on the silencing of oncoproteins and oncomirs and exploring its interventional influence on the genomic instability in prostate cancer. He has few national publications. Scientific interests: involvement of ATM in safeguarding against genomic insult, prostate cancer therapeutic interventions.

Abuzar Kaleem
E-mail: abuzarkaleem@yahoo.com

Mr. Abuzar Kaleem has received his B.Sc. (Hons) degree in biochemistry from the University of Lahore. He has presented his research findings in multiple national conferences. Research interests: transient receptor potential channel emerging role in prostate cancer.

Muhammad Khalid Javeed, M.Sc.
E-mail: khalid_8627@yahoo.com

Mr. Khalid javeed has done his Masters in Molecular biology from the University of Lahore. He has been under supervision of Ammad Ahmad Farooqi in prostate cancer biology lab. He has worked as scientific officer in Shaukat Khanum memorial, Cancer Hospital. Research interests: cancer biology.

Qaisar Mansoor, M.Sc.
E-mail: qmibge@gmail.com

Mr. Qaisar Mansoor has received his M.Sc. degree from from Quaid-i-Azam University, Islamabad. Serving as Scientific Officer at the Institute of Biomedical and Genetic Engineering, Islamabad since 2006. He has few national publications. He has presented his research findings in various national and international conferences. Research interests: cancer genetics, metabolic molecular anomalies.

Assist. Prof. Shahzad Bhatti, M.Phil., Ph.D. Student
E-mail: drshahzadbhatti@yahoo.com

Dr. Shahzad Bhatti is an eminent scholar. He holds multiple M.Phil. degrees in Pathology (University of Veterinary and Animal Sciences Lahore), Molecular Biology (Center of Excellence in Molecular Biology, University of the Punjab) and criminology (University of the Punjab, Lahore) which are some of renowned educational institutions of Pakistan. He received his degree in veterinary medicine from University of Agriculture, Faisalabad. He is Member of World Academy of Science, Engineering and Technology (WASET). Currently he is engaged in finalization of his Ph.D. project. He has few national publications. Since 2008 he is an assistant professor in the Molecular Biology Department of the University of Lahore. He is also a visiting faculty member of GCU (Forensic chemistry department), Lahore. Research interests: translational medicine, factors underlying mitochondrial desensitization in oncogenesis.