**Biological Regulatory Network (BRN) Analysis and Molecular Docking Simulations to Probe the Modulation of IP3R Mediated Ca2+ Signaling in Cancer**

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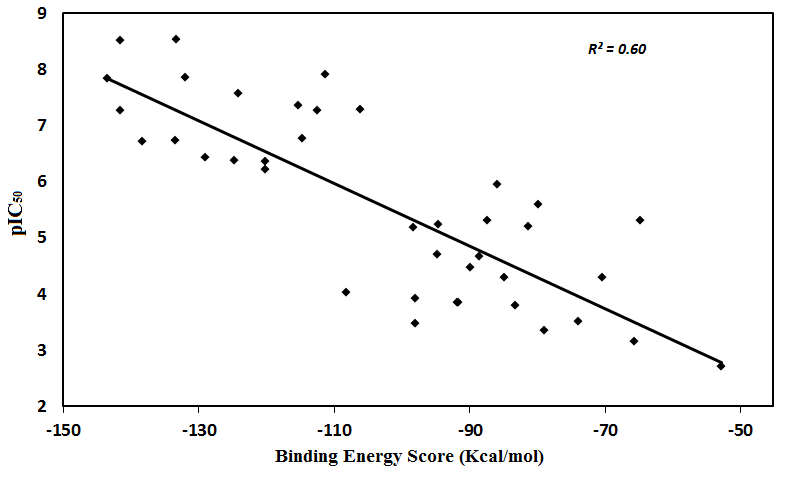
1. **Materials and Methods:**

In order to construct a biological regulatory network (BRN) we refined and build the topology of available information about interactive data from literature [[1-6](#_ENREF_1)] and Kyoto Encyclopedia of Genes and Genomes (KEGG) databank. The interactive oncogenes and proto oncogenes are summarized in supplementary **Table S1**.

**Table S1: Onco-proteins and tumor suppressor proteins interacting with IP3R**

|  |  |  |
| --- | --- | --- |
| **Protein** | **Function** | **Ref** |
| AKT | Phosphorylate IP3Rs and have negative effect on Ca2+ dependent apoptosis. | [[7-10](#_ENREF_7)] |
| mTOR | Controls phosphorylation of IP3R regulated by AKT and promotes Ca2+ uptake. | [[9](#_ENREF_9), [11](#_ENREF_11), [12](#_ENREF_12)] |
| PTEN | De phosphorylates IP3Rs and Sensitizes cells to Ca2+ dependent apoptosis. | [[2](#_ENREF_2), [13](#_ENREF_13), [14](#_ENREF_14)] |
| PML | Forms a complex with AKT and PP2A and promotes ER-mitochondria Ca2+ transfer and apoptosis. PP2A suppresses AKT-mediated IP3R3 phosphorylation. | [[15](#_ENREF_15), [16](#_ENREF_16)] |
| p53 | It regulates Ca2+ release in IP3R3 via interacting with SERCA pumps. | [[17](#_ENREF_17), [18](#_ENREF_18)] |
| Bcl-2 | It limits the transfer of pro-apoptotic Ca2+ signals and can induce leakage of Ca2+ from ER to the mitochondria by acting on both organelles. | [[19-21](#_ENREF_19)] |
| Bcl-XL | Enhances Ca2+ oscillations and increases basal IP3R function. When IP3 level lower down it sensitizing the channels to IP3R, thus act as pro-survival protein. | [[22](#_ENREF_22), [23](#_ENREF_23)] |
| BAD | It binds with Bcl-XL when dephosphorylated and antagonizes its anti- apoptotic effect. Thus activates apoptosis by subsequent activation of Bax/Bak. | [[24](#_ENREF_24), [25](#_ENREF_25)] |
| CaN | Ca2+ signals activates CaN that dephosphorylates NFAT. This leads NFAT to translocate into the nucleus and transcription activated. It involves in cell cycle progression. | [[26-30](#_ENREF_26)] |
| CaMKII | Involves in Ca2+ signaling pathways by interacting with Filamin and involved in cell cycle progression. | [[28](#_ENREF_28), [29](#_ENREF_29), [31](#_ENREF_31)] |

1. **Results & Discussion:**

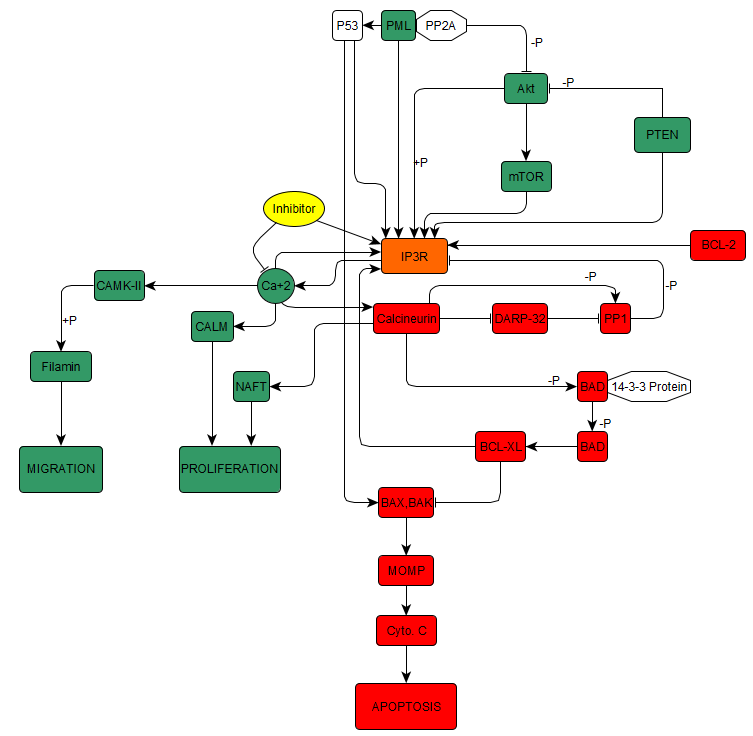


**Figure S1:** Correlation plot of the binding poses of dataset of IP3R modulators showing better correlation (R2) between binding energy score and pIC50 value.

1. **Biological Regulatory Network (BRN):**

IP3R mediated Ca2+ siganlingis responsible for cell proliferation and apoptosis. In proliferation, different binding proteins are congregated towards the IP3R by Calmodulin (CaM) [[32](#_ENREF_32)], initiating the downstream signaling cascade of Ca2+/CaM-dependent phosphatase and Ca2+/CaM-kinases (CaMK) [[33](#_ENREF_33)]. Furthermore, Calcineurin (CaN), upon activation by Ca2+, triggers the nuclear factor of activated T-cells (NFAT) pathway [[30](#_ENREF_30), [34](#_ENREF_34)] that also has a significant role in initiation of proliferation by progressing cell cycle phases [[35-37](#_ENREF_35)]. Any disruption in IP3R mediated Ca+2 level creates stress and activates pro-survival response (i.e. autophagy) [[38](#_ENREF_38), [39](#_ENREF_39)]. However, these cell survival responses (autophagy) may lead towards the cell death if intureption prolonged and IP3R fails to regain its normal function. Successively, the intrinsic apoptotic pathway initiated in response, that is provoked by the mitochondrial outer membrane permeabilization (MOMP) induction [[40](#_ENREF_40), [41](#_ENREF_41)]. This induction regulates the multi domain pro-apoptotic protein family BCL-2 along with BAX and BAK [[40](#_ENREF_40), [42](#_ENREF_42), [43](#_ENREF_43)], which is considered crucial in regulation of cell death [[44](#_ENREF_44), [45](#_ENREF_45)].

Conversaly, an authoritative long term growth signal response is seen in cancer cells by reprogramming of host energy metabolism [[46](#_ENREF_46), [47](#_ENREF_47)]. In many cancer types, Bcl-2 targets the central modulatory domain of IP3R, thus arrests the IP3R activity in pro-apoptotic Ca2+ release events [[25](#_ENREF_25), [48](#_ENREF_48)]. Whereby, overexpression of Bcl-2 results in reduction of Ca2+ level within ER followed by reduced fragmentation in mitochondria that can initiate the apoptosis [[49](#_ENREF_49)]. Furthermore, tumor suppressors like PTEN and PML, and oncogenes like Akt play an important role in development of cancer via modulation of IP3R dependent Ca+2 signaling [[47](#_ENREF_47), [50](#_ENREF_50)].



**Figure S2:** Dynamic simulation of biological regulatroy network (BRN) showing the role of IP3R and calcium in proliferatiion and apoptosis signaling casecade. The entities with green color shows the proliferation in normal cells. In normal cells, the proliferation pathway activates and apoptosis siganls remains at minimal level of expression. While the entities with red color shows the apoptosis when inhibitor is added in the BRN. Here, if a stress is created due to insufficient calcium pool or an ihibitor added to normal or cancerous cell that modulates the IP3R activity, as a result, the apoptosis induces by reducing the proliferation signal peaks in BRN.

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