Calcium-binding protein antagonists as therapeutic and diagnostic tool in tumors

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My story

2015-2016	• laboratory of physiology on thesis “influence of some food additive cocktail effect on rats behavior and on internal organs.”department in TSU.
2016-2018	• After physiological experiments I started Morphology examinations at Department of Morphology.
23.08. 2018	• 8th GGSWBS -2018
02. 2019	• Internship in Juelich
08.09.2019	• Internship in Juelich

“All roads lead to Rome”

“All pathways lead to Calcium”

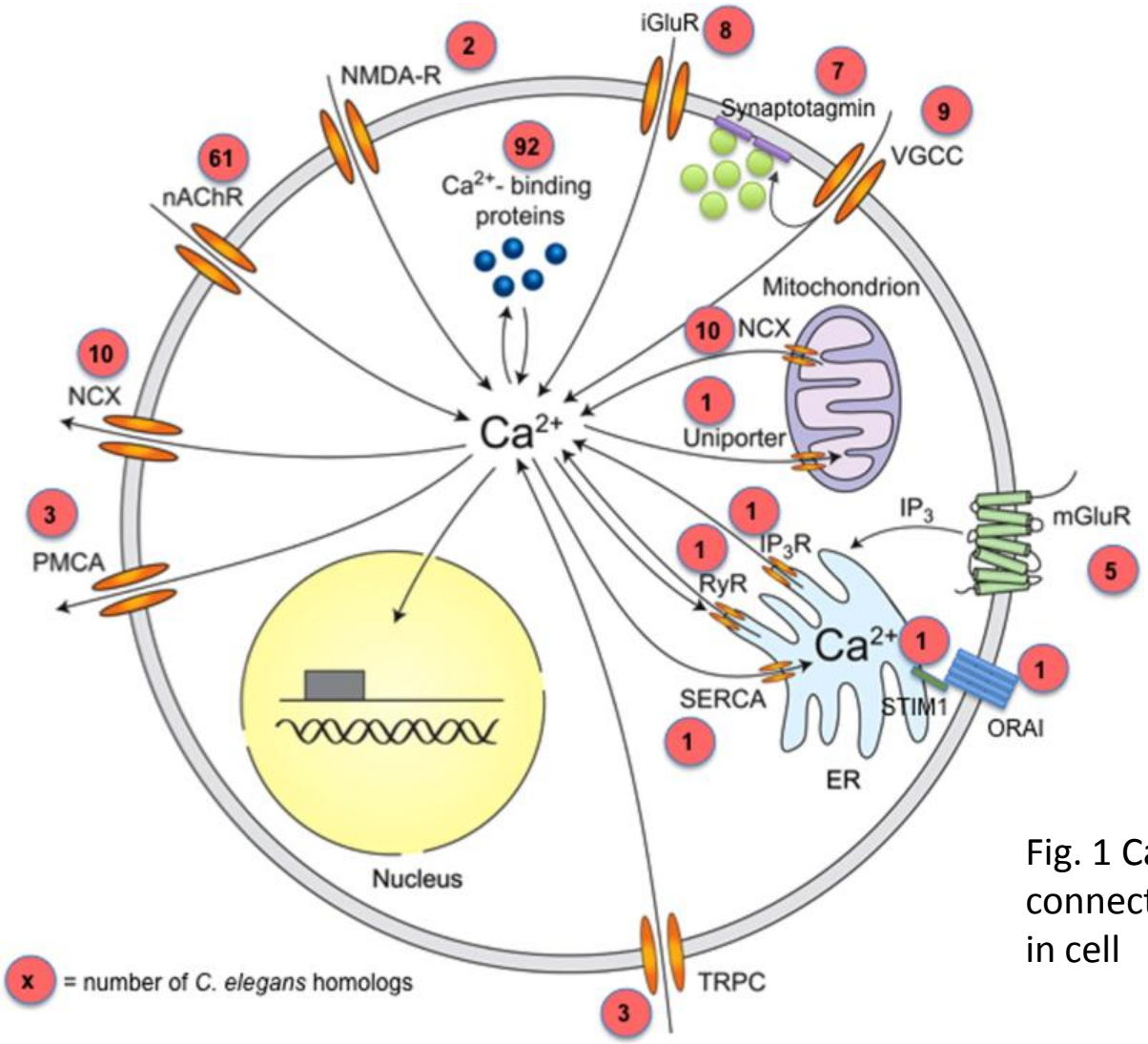


Fig. 1 Calcium connected processes in cell

Calcium -binding proteins

- Most common calcium-binding structural motifs is EF-hand, theyll deffined helix-loop-helix structural domain.
- parvalbumin, S100, calmodulin, calcineurin

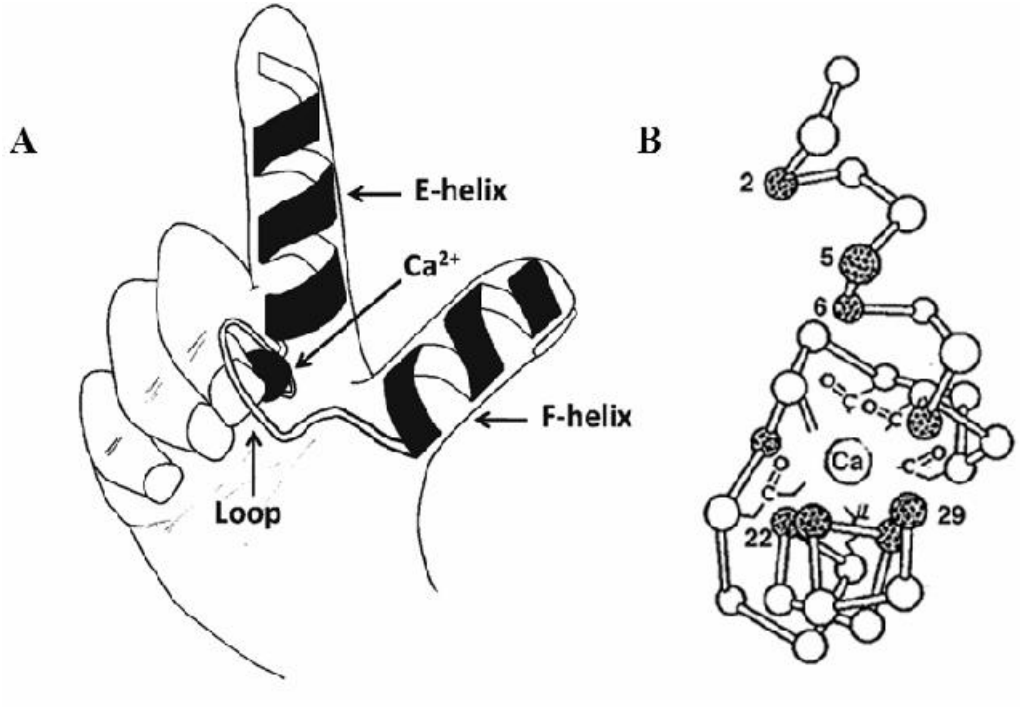
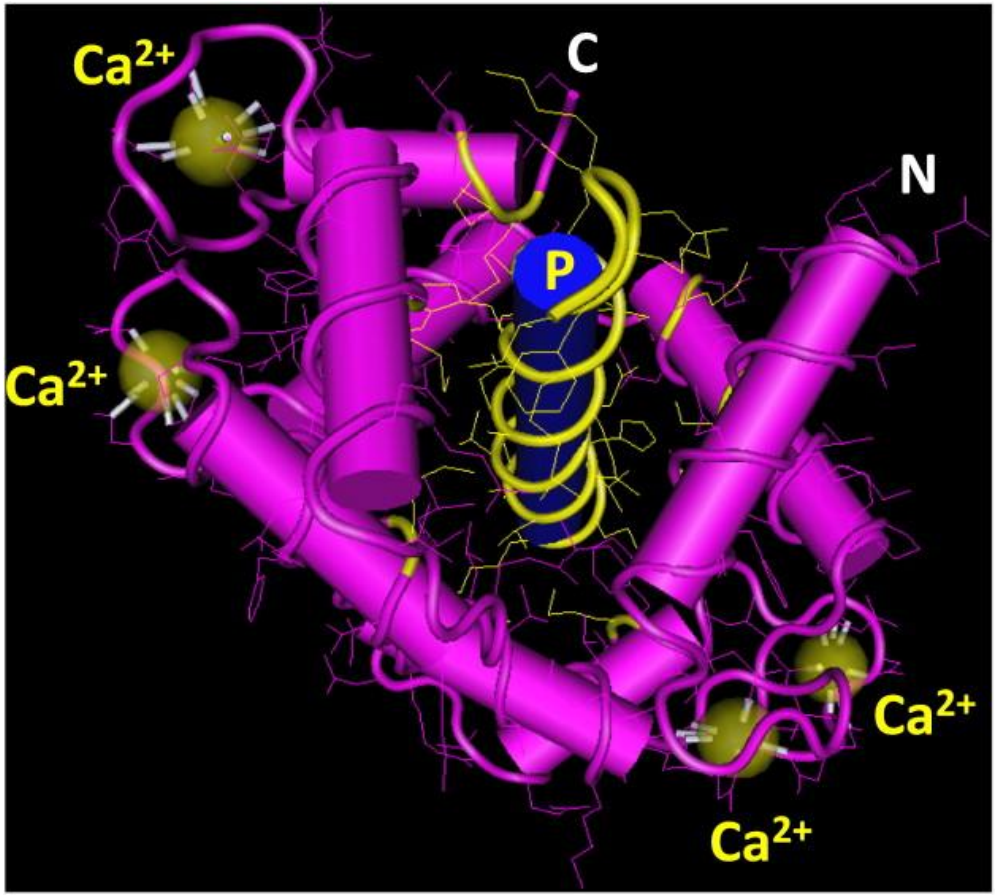


Fig.2 A - Model of EF-hands; B – Ca binding protein.

What is a Calmodulin?



- Calmodulin (CaM), a calcium (Ca^{2+})-trigger protein with four EF hands, is highly conserved molecule.

Fig. 3 Interaction of calmodulin with a CaMK-I peptide. Model of Ca^{2+}/CaM (pink) collapsed around a peptide (blue/yellow barrel labeled P) corresponding to the CaM-binding site of CaMK-I. CaM methionine residues are labeled in yellow. The N- and C- termini of CaM and Ca^{2+} ions are indicated.

Holo and Apo Calmoduline

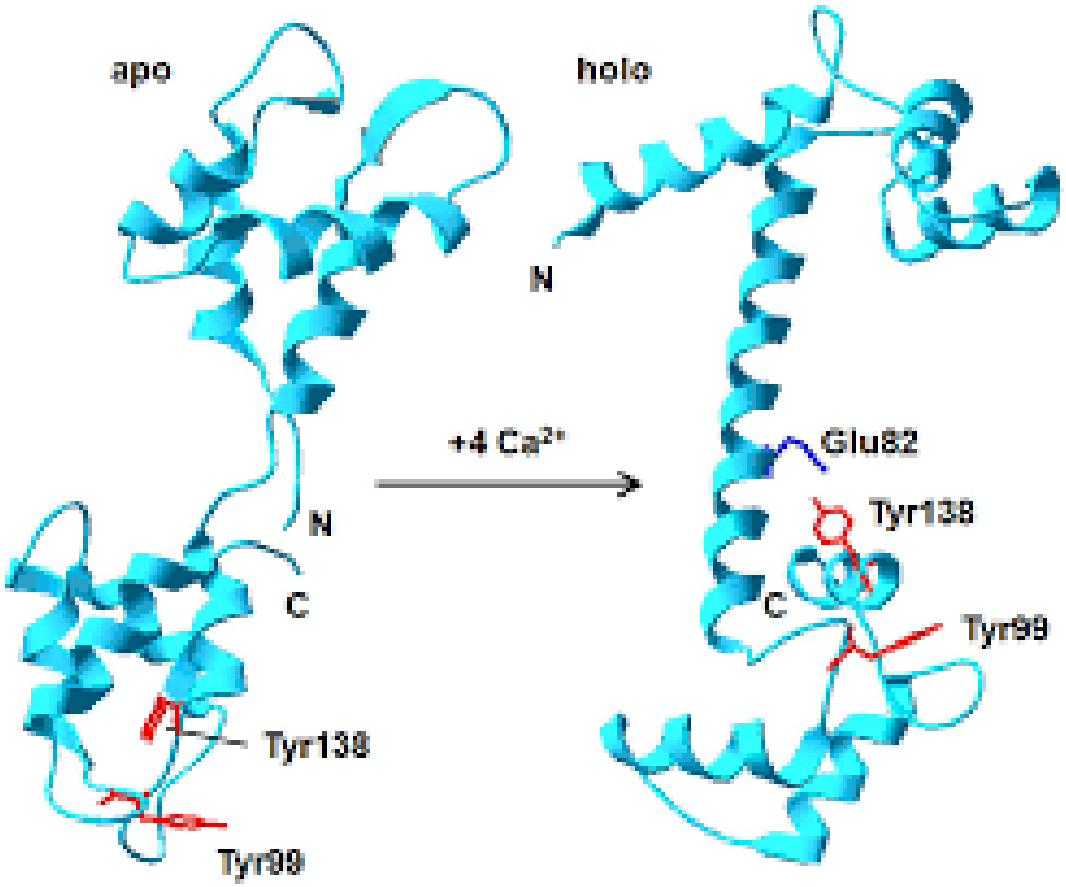


Fig.4 Reaction of binding Ca2+ ions.

Overview of CaM functions in Cell

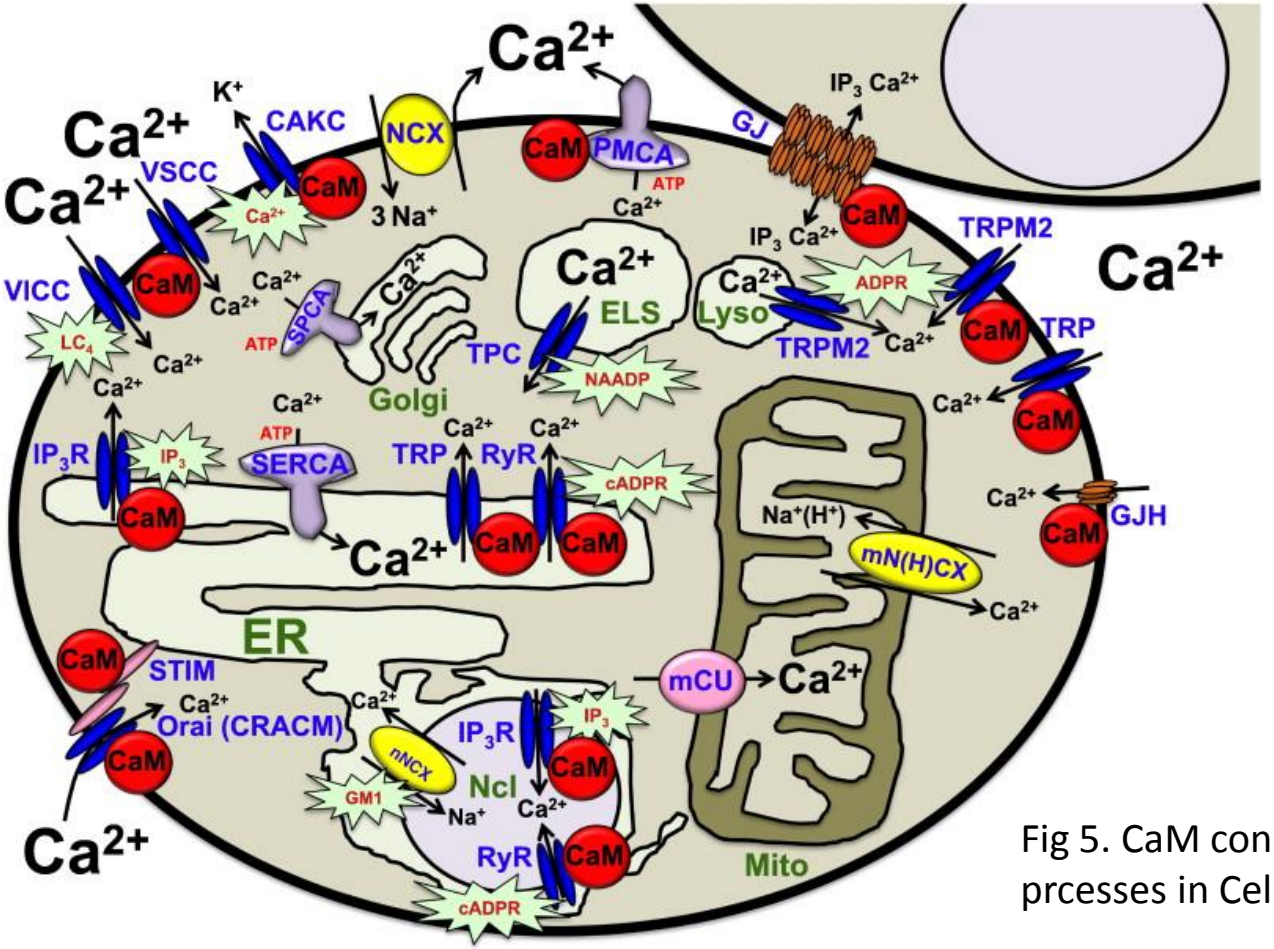
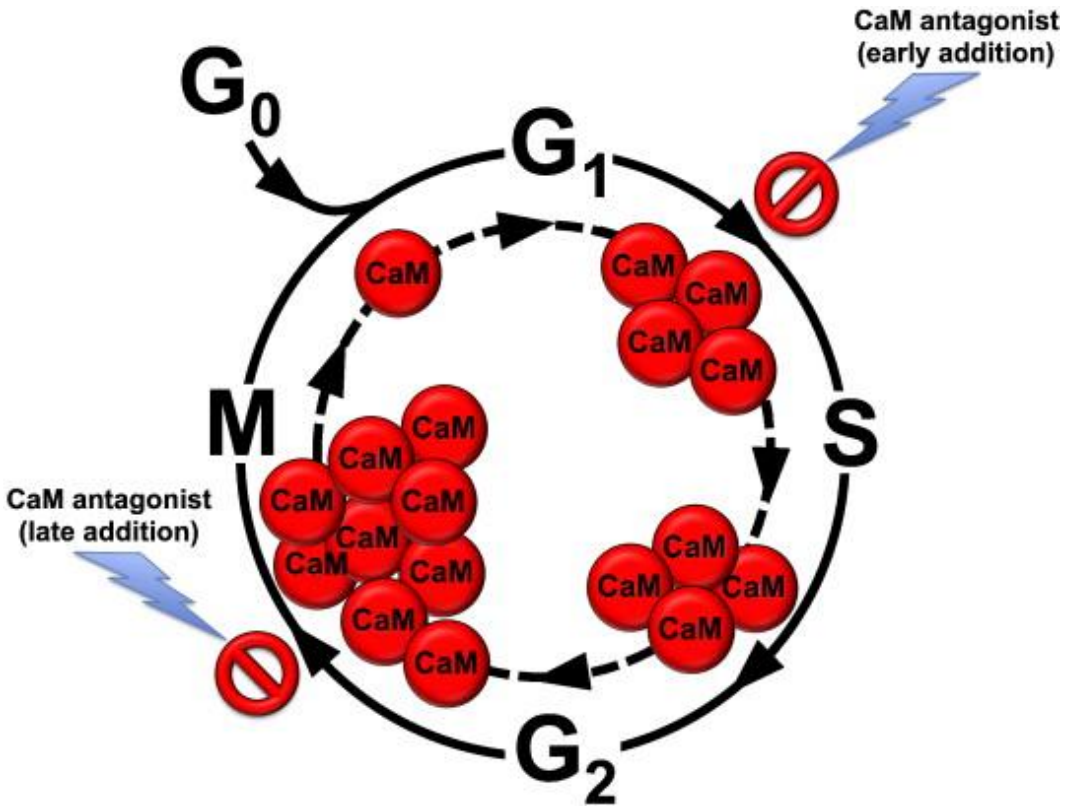


Fig 5. CaM connected prcesses in Cell

Proliferation



Calmodulin levels during cell cycle progression. The scheme depicts the increment of calmodulin (CaM) expression at the different phases of the cell cycle; and the two major points of cell cycle arrest by CaM antagonists added at the indicated phases.

Fig. 6

CaM – dependent Kinases

CaM-kinase	Selected comments
CaMK-I	Controls the cell cycle (G_0/G_1)
CaMK-II	Activates the MAPK pathway Controls the cell cycle (G_1/S and G_2/M)
CaMK-III	Phosphorylates eEF2 during active cell proliferation
CaMK-IV	Controls cell proliferation phosphorylating transcription factors (Sp1, CREB, ATF1)
CaMKK	Controls cell proliferation by phosphorylating CaMK-I/IV
CASK	Contains an N-terminal CaMK domain Negatively regulates the cell cycle upregulating $p21^{Cip1/Waf1}$
DAPK	Activates cell growth and protein synthesis by phosphorylation of ribosomal protein S6 via the EGFR/MAPK(ERK)/DAPK pathway
PNCK	Closely related to CaMK-I. Downregulated upon entry in cell

CaM in Tumour Cells

- The level of CaM in many tumor cells appears to be higher as compared to cells from normal tissues.
- A positive correlation between the rate of cell growth and the degree of tumor malignancy with the level of cellular CaM has been postulated.
- the MTS1 gene, encoding the Ca2+-binding protein metastasin 1 (also denoted S100A4), that is highly expressed in tumor cells with high metastatic potential
- Ca2/CaM plays a crucial role in angiogenesis. Ca2+/CaM activates HIF-1 an consequently induces the expression of pro-angiogenic factors such as VEGF.

- CaM Antagonists can inhibit the proliferation of metastatic and tumorous cells.

Effects of distinct CaM antagonists on tumor cells.

CaM antagonist	Tumor/cell/tissue	Origin	Effect
B859-35	Neuroendocrine lung tumors	Hamster	Inhibits tumor growth *in vivo*
	Lung carcinoid NCI-H727, adenocarcinoma NCI-H322 and NCI-H358 cells	Human	Inhibits cell proliferation (at 0.001 pM–100 nM)
Berberine[a]	Hepatocellular carcinoma Bel7402 cells	Human	Arrest the cell cycle at G_1 W-7 and TFP pontentiate its action. Inhibits CaMK-II and MEK1, and p27 degradation (CaM-unrelated effects observed)
Calmidazolium (R24571)	Breast adenocarcinoma MCF-7, T47D, ZR-75-1 cells	Human	Inhibits cell proliferation at the early to mid G_1 phase of the cell cycle. Induces apoptosis-like cell death potentiated by antiestrogen drugs
	Serous cystadenocarcinoma ovary cells	Human	Inhibits cell proliferation and prevents EGF binding to its receptor
	Pituitary tumor GH_3 cells (ER-positive)	Rat	Induces apoptosis-like cell death potentiated by antiestrogen drugs
	ASV-transformed cells	Rat	Inhibits cell cycle at late G_1
	Ehrlich ascites tumor cells	Mouse	Inhibits protein synthesis
	Astrocytoma C6 cells	Rat	Inhibits cell proliferation
Chlorpromazine	Leukemia cells	Human and mouse	Inhibits cell growth and clonogenicit
	Astrocytoma C6 cells	Rat	Inhibits cell proliferation
Compounds 1, 2 and 3[b]	Breast adenocarcinoma T-47D, MCF-7B,	Human	Inhibits cell proliferation

Ca/CaM Antagonist HBC inhibits Angiogenesis and downregulates Hypoxia-inducible factor

- A curcumin derivative, HBC 4-{3,5-bis-[2-(4-hydroxy-3-methoxyphenyl)ethyl]-4,5-dihydropyrazol-1-yl}benzoic acid

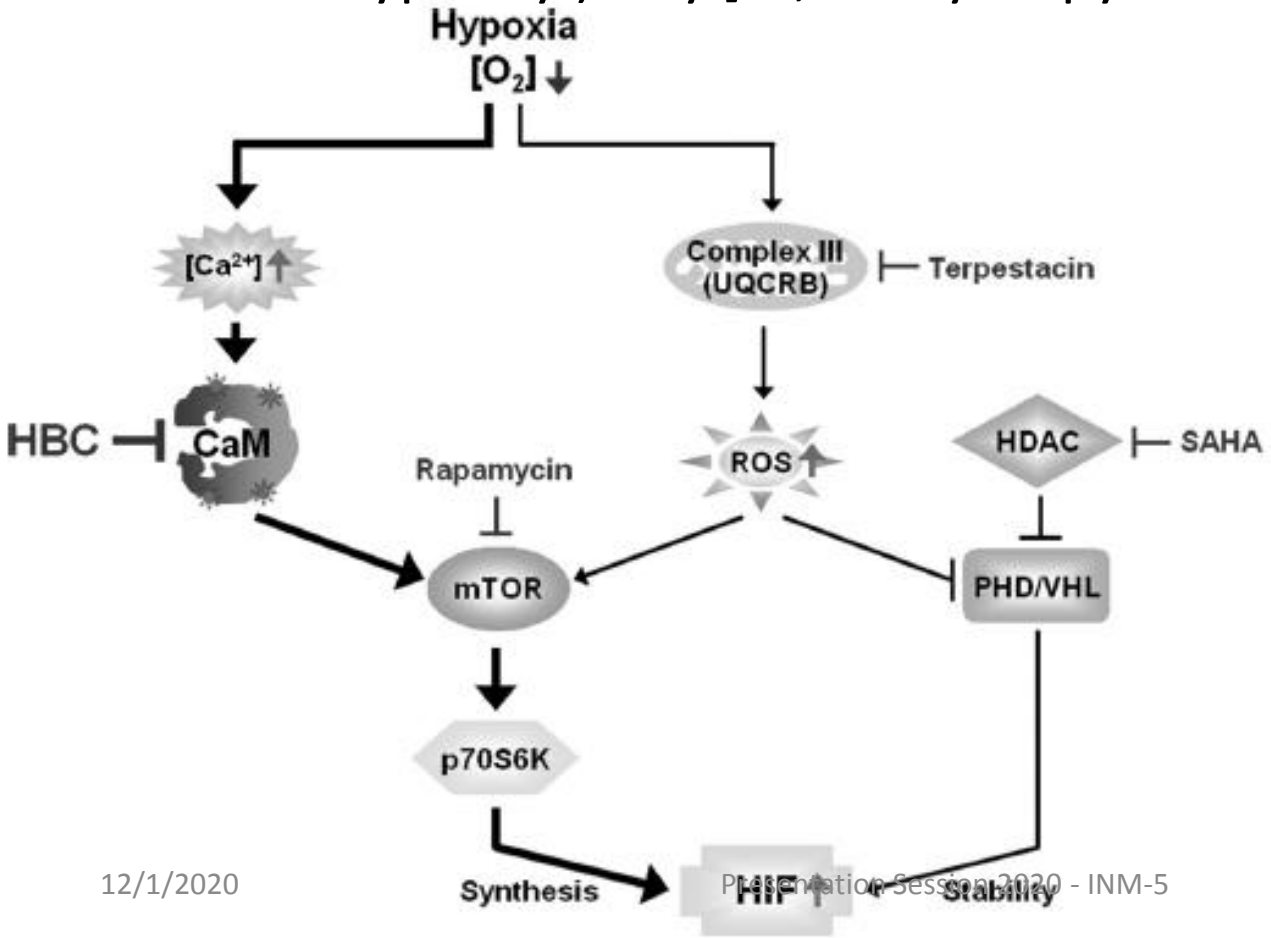
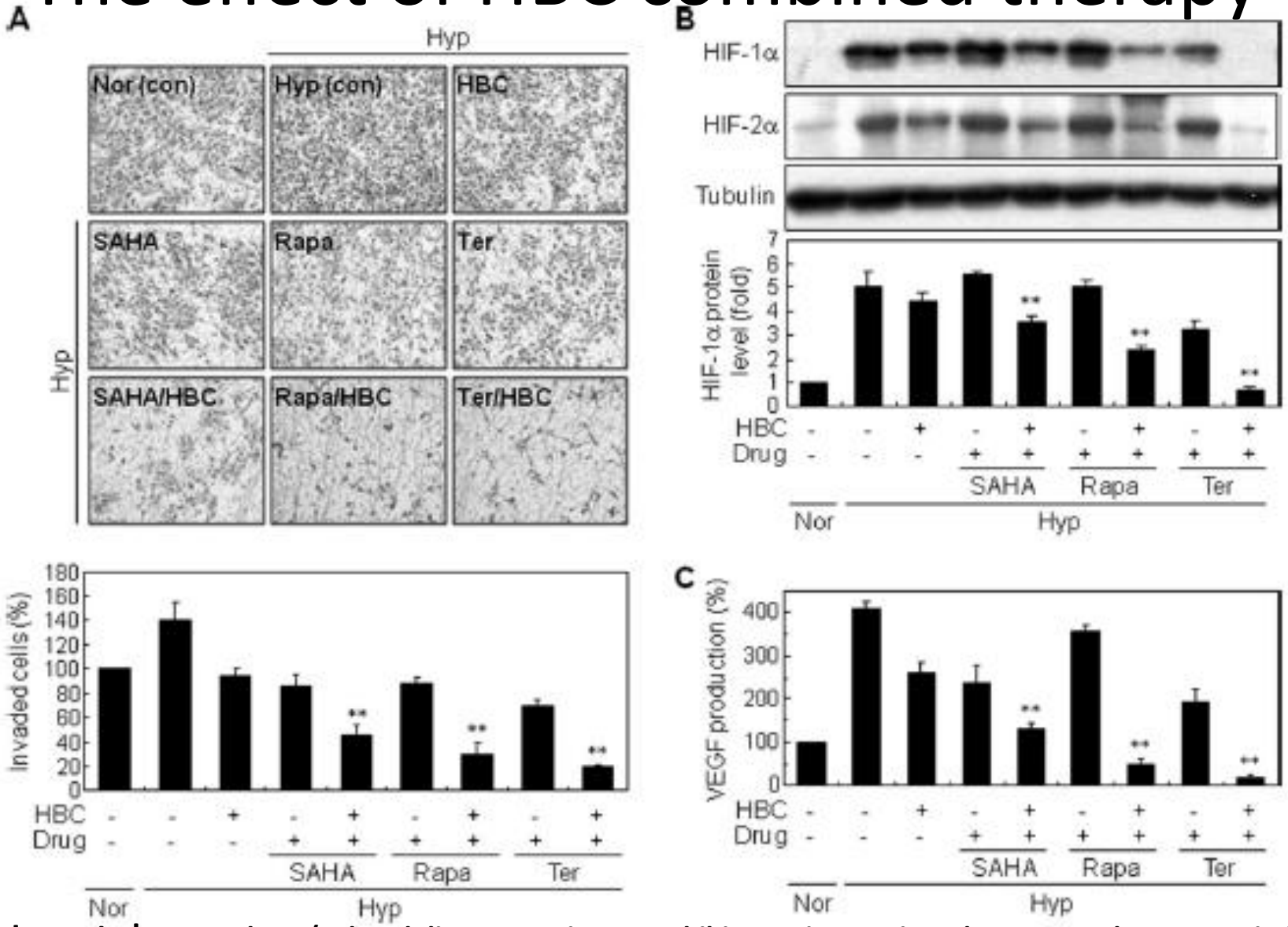


Fig. 7
mTOR- mamalian target of Rapamycin
ROS- Reactive oxygen species
HDAC- Histone deacetylase
SAHA – suberanilohydroxamic acid
UQCRB- Ubiquinol Cytochrome C Reductase Binding Protein
VHL- von Hippel- Lindau tum. Suppresor
PHD – Plant Homeodomain
p70S6K- ribosomal protein S6 kinase

The effect of HBC combined therapy



Hye Jin Jung et al. A Novel Ca2/Calmodulin Antagonist HBC Inhibits Angiogenesis and Down-regulates Hypoxia-inducible Factor.

- Today, calcium binding proteins and related them aspects are considered promising new chapter in future diagnostic and therapeutic methods.
- Research about this thesis are going on fast.
- Not only peptides are promising tool in PET diagnostic but its associated molecules too.
- Perhaps, this kind of molecules gives us chance to merge diagnostic and therapeutic process.

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The interactome and spatial redistribution feature of Ca2+ receptor protein calmodulin reveals a novel role in invadopodia-mediated invasion

Tao Li, Li Yi, Long Hai, Haitheyn Ma, Zhennan Tao, Chen Zhang, Iruni Roshanie Abeysekera, Kai Zhao, Yihan Yang, theyi Wang, Bo Liu, Shengping Yu, Luqing Tong, Peidong Liu, Meng Zhu, Bingcheng Ren, Yu Lin, Kai Zhang, Cheng Cheng, Yubao Huang & Xuejun Yang

Cell Death & Diseasevolume 9, Article number: 292 (2018)

- **Hye Jin Jung et al. A Novel Ca2/Calmodulin Antagonist HBC Inhibits Angiogenesis and Down-regulates Hypoxia-inducible Factor. 2010.** *From the Chemical Genomics Laboratory, Department of Biotechnology, Translational Research Center for Protein Function*
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Acknowledgements

- Thanks to every person, who is a friend and supporter of georgian – german science bridge.
- My Supervisors Ekaterine Mitaishvili and Diana Dzidziguri

THANK YOU FOR YOUR ATTENTION

QUESTIONS???

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