SHINE model dynamic behavior analysis

The present analysis is based on the attractors reached from 100,000 initial states (Out of the 2^{205} possible). Which we interpreted as observable endothelial behaviors corresponding to quiescent or dysfunctional pulmonary microvascular endothelial cells (ECs). These atractors where also include an interpretation of glycocalyx damage and oxidative stress.

Nathan Weinstein 18/12/2024

Conditions that promote EC quiescence

- High and constant shear stress caused by laminar blood flow.
- ANG1/TIE2 signaling sustained by periciytes
- Canonical WNT signaling
- Normoxia
- Low/No inflammation
- Low/No oxidative stress
- ADRB2 signaling
- **We found 398 quiescent attractors**

Active in all quiescent states

- AA, **AC**, ADD, AF6, AKAP, *a_ketoglutarate,* ArhGAP29, **Bcatenin**, ***BH4***, Bkca, **cAMP, CDH5, cGMP**, **Claudin1_3, Claudin5**, COX1, CREB3, Cav, Cofilin, DAG, **eNOS**, EPAC1, G12, GEFH1, Gq, Gs, ***heparan_sulfate***, ***HIF1B***, IP, IP3, IRAG, K_hyperpolarisation, KRIT1, ***LArg, LCit,*** lysophospholypids, **MLCP**, **NO**, **O2**, **Occludin**, OXGR1, **p120_catenin,** PAC, PGH2, **PGI2,** PKA, PKGIB, PLA2, PLC, PPARB, *propionylLcarnitine,* PTGIS, ***Phospholypid***, RAC1, RADIL, RAP1, RASIP1, RhoGDIa, **sGC,** SERCA. SLC2A1, **Syndecan1,** TBXAS1, TIAM1, TLE1, TP, TXA2, VASP, VAV2, VHL, ZNF185, **ZO1, barrier_function**
- **From our definition of quiescence**
- **Assumed or experimentally observed parameters**
- **Key molecules**
- Most of the other molecules are involved in PGI2 or cAMP signaling or synthesis

Active in some states

- ACTG1, **ADRA1, ADRA2**, **ADRB**, **AKT1**, **ANG1**, AP1, ARRB1, AXL, CALD1, CDC42, DAAM1, DKK1, **DVL1, ERK1, ETS**, **EPI**, **FYN**, **FZD5, FZD7**, Fe2, GAB1, GRB2, **Gi**, HCO3, IGF, **KLF2, LTyr, LRP5**, **Lactate**, MAPK14, MAPKAPK2, MKK3, **MMP2_9_10,** NRF2, **NE**, PAK, **PDE4D**, **PECAM1**, PHD1, PI3K, **PIEZO1**, PIP2, PIP3, RAS, ROR2, sFRP, **S1PR1**, SHP2, **S1P**, SOS1, **SUCNR1**, **ShearStress**, **Succinate**, **TIE2**, **TRPV4**, VegfA, **WNT5a**, **WNT7a**
- **Molecules that promote quiescence, might be sufficient but not necessary, contributing to the robustness of EC function.**
- **Molecules that inhibit quiescence.**
- **Molecules that have a context-dependant effect catecholamines are discussed in the next slide. The calcium channels might contribute to the basal Ca concentration that allows eNOS and VE-cadherin function or cause MIC-mediated EC contraction.**

Catecholamines can promote quiescence

- The α2A, αC \cite{xu2018adrenoceptor}, β1, and β2 \cite{mcgee2024beta} adrenergic receptors are highly expressed in HPMECs. α2 adrenergic receptor activity preserves VE-caherin expression and endothelial barrier function in HPMECs exposed to TNF-α \cite{xu2018adrenoceptor}. Similarly, β2 adrenergic receptor signaling attenuates Thrombin-induced increases in HPMEC barrier permeability \cite{mcgee2024beta} by increasing the concentration of cAMP \cite{sorriento2011adrenergic}. (Added to a new subsection in the quiescence section of the manuscript).
- The abundance of α2 and β2 suggests that a small dose of catecholamines would likely increase cAMP and NO preserving endothelial barrier function. However, α1B is also highly expressed \cite{mcgee2024beta}. I do not know how α2 adrenergic receptor activity preserves VE-caherin expression.

Dose-dependent barrier permeability caused by catecholamines

- ADRA1A, ADRA1B, and ADRA1D through Gq increase MLC activity resulting in EC contraction and increase Ca2+ concentration in the cytoplasm which inhibits AC6 decreasing cAMP.
- ADRB2 can switch to Gi leading to AC inhibition and SRC activation.
- López García de Lomana, A., Vilhjálmsson, A. I., McGarrity, S., Sigurðardóttir, R., Anuforo, Ó., Viktorsdóttir, A. R., ... & Rolfsson, Ó. (2022). Metabolic response in endothelial cells to catecholamine stimulation associated with increased vascular permeability. International journal of molecular sciences, 23(6), 3162.

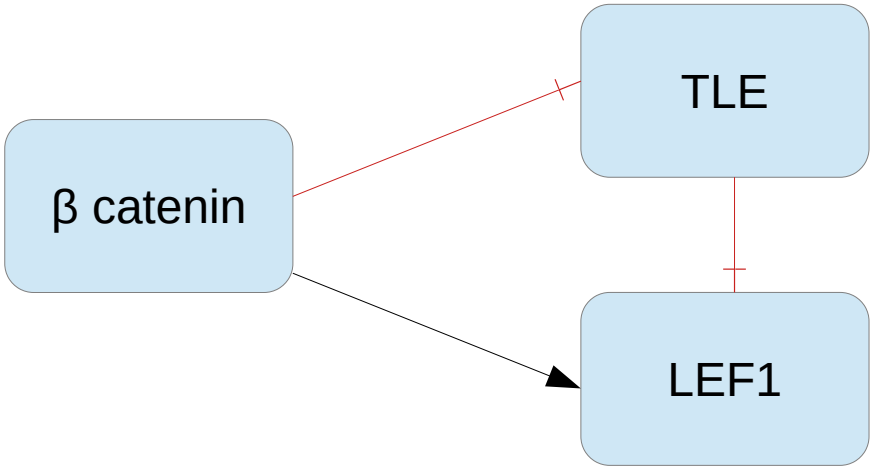
Ways to simulate the dose-dependent effect of catecholamines

- The current model is a valid approach because it emphasizes how catecholamine signaling interacts with other pathways that can amplify a certain behavior. For instance PGI2 can also activate AC6 increasing cAMP-mediated endothelial barrier stabilization. Other conditions like hypoxia or low flow might promote the permeability increases associated with high doses of catecholamines, those conditions are analyzed later in this presentation.
- We can simulate the dose dependence by using ADRA1, ADRA2, and ADRB as inputs, then a low catecholamine dose is represented by ADRB activity only, and a high dose of catecholamines is represented by the simultaneous activity of all receptors.
- Petri nets or ODE models can represent this with more detail. I propose first analyzing carefully the current model. And only then decide which additional approach to try.

Inactive in all quiescent attractors.

- ACTB, ANG2, ASFSF2, AXINcomplex, Axin2, BTRC, CAM, CEBPB, CPI17, CREB1, Ca2, Calcineurin, DDC, DO, DBH, FAK, FOXO1, FilGAP, H2O2, HIF1, HIF1A, HIFIAh, HIF1Au, HSP27, IL6, IL6R, IL6ST, Integrin, IP3R, JAK, LDOPA, LEF1, mDia, MEK, MLC, MLCK, MMP24_25, NCK, NFAT, NfkB, NOX2, NOX4, NOX5, NPR1, Superoxide, p47phox, PKC, PLVAP, PNMT, pSTAT3, Phospholamban, RAF, RHOA, ROCK, RRAS, sAC, SOD, SRC, TF, TH, TIE1, TSAd, VEGFA, VEGFR11, VEGFR12, VEGFR1s, VEGFR22, VegfR1, VegfR2, vWF, oxidative_stress, glycocalyx_shedding, endothelial_dysfunction
- By definition
- Calcium signaling and cell contraction
- Glycocalyx shedding
- IL6 Signaling
- CREB signaling and catecholamine production
- ANG2 and VEGF signaling
- Inhibits or is inhibited by canonical WNT
- Hypoxia and oxidative stress

LEF1



β catenin forms a complex with LEF1 to activate the transcription of WNT targets. TLE1 binds LEF1 preventing it from binding β catenin (https://www.cell.com/AJHG/fulltext/S0960-9822(06)02259-7, https://link.springer.com/article/10.1186/1471-2407-9-159)

Old: TLE1, Bcatenin
New: TLE1, JBcatenin

Barrier function

- **barrier_function, ZO1 & CDH5 & (Claudin1_3 | Claudin5) & Occludin**
- **8340 attractors with barrier function, about 21 x the number of quiescent attractors**
- **This implies robustnes of the barrier function to oxidative stress, glycocalyx shedding, or endothelial dysfunction.**

Active in all ECs that can form functional barriers

- **AA**, **AC, ADD, AF6, AKAP**, **a_ketoglutarate**, **ArhGAP29**, **Bcatenin**, **cAMP**, **CDH5, Claudin1_3, Claudin5**, **COX1**, **CREB3**, **Cofilin**, **DAG**, **EPAC1**, **G12**, **GEFH1**, **Gq**, **Gs**, **HIF1B**, **IP**, **IP3**, **KRIT1**, **lysophospholypids**, **MLCP**, **Occludin**, OXGR1, **p120_catenin, PAC**, **PGH2, PGI2**, **PKA**, **PLA2**, **PLC, PPARB, propionylLcarnitine**, **PTGIS**, **Phospholypid**, **RAC1, RADIL, RAP1, RASIP1**, **RhoGDIa**, **SLC2A1**, **TBXAS1**, **TIAM1**, TLE1, **TP, TXA2**, **VASP, VAV2**, **VHL**, **ZNF185**, **ZO1, barrier_function**.
- **By definition.**
- **Model parameters.**
- **Other AJ, TJ, and FA components.**
- **Associated to PGI2 synthesis or signaling.**
- **CAMP signaling.**
- **Prevents MLC activation and EC contraction.**
- TLE1 see slide 9.

Active in some functional EC barriers

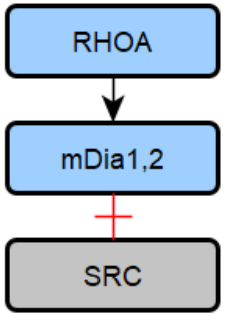
- ACTG1, **ADRA1, ADRA2, ADRB**, **AKT1**, **ANG1**, AP1, ARRB1, AXL, **BH4**, Bkca, CALD1, CDC42, **cGMP**, **CREB1**, Cav, DAAM1, DDC, DKK1, DO, DVL1, DBH, **eNOS**, ERK, ETS, **EPI**, FYN, **FZD5, FZD7**, Fe2, GAB1, GRB2, **Gi**, **H2O2**, HCO3, heparan_sulfate, **HIF1, HIF1A, HIFIAh, HIF1Au**, HSP27, IGF, **IP3R**, IRAG, K_hyperpolarisation, **KLF2**, LArg, LCit, LDOPA, LTyr, LRP5, Lactate, MAPK14, MAPKAPK2, MKK3, **MMP2_9_10, MMP24_25, NFkB**, **NO, NOX4**, NPR1, NRF2, **NE**, **O2**, **Superoxide**, PAK, **PDE4D**, **PECAM1**, PHD1, PI3K, **PIEZO1**, PIP2, PIP3, PKGIB, PNMT, Phospholamban, RAS, ROR2, **sGC**, sFRP, **S1PR1**, SERCA, SHP2, **S1P**, SOD, SOS1, SUCNR1, **ShearStress**, Succinate, **Syndecan1**, TH, **TIE2**, **TRPV4, VEGFA**, VEGFR11, VEGFR1s, VegfA, VegfR1, **WNT5a, WNT7a**, **oxidative_stress, glycocalyx_shedding, endothelial_dysfunction**
- **Increase permeability**
- **Decrease permeability**
- **The effect of the catecholamines depends on the dose, low decreases permeability, high increases permeability**

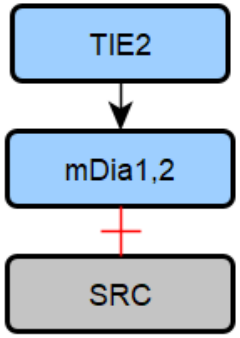
Molecules inactive in ECs with normal barrier function

ACTB, ANG2, ASFSF2, AXINcomplex, Axin2, BTRC, CAM, CEBPB, CPI17, Ca2, Calcineurin, FAK, FOXO1, FilGAP, IL6, IL6R, IL6ST, Integrin, JAK, ~~LEF1~~, ~~mDia~~, MEK, MLC, MLCK, NCK**, NFAT, NOX2, NOX5, p47phox, PKC, PLVAP, pSTAT3, RAF, RHOA, ROCK, RRAS, sAC, SRC, TF,** TIE1**, TSAd, VEGFR12, VEGFR22,** VegfR2**, vWF**

- **Cytoskeleton components associated with remodeling and barrier destabilization**
- **Increase permeability**
- **Inhibit or inhibited by WNT**
- **IL6 signaling**
- **Oxidative stress**
- **Ca2 signaling and EC contraction**
- **Hemostasis**
- **Not accurate LEF1 see slide 9, mDia next slide**

Pathways that need some thought





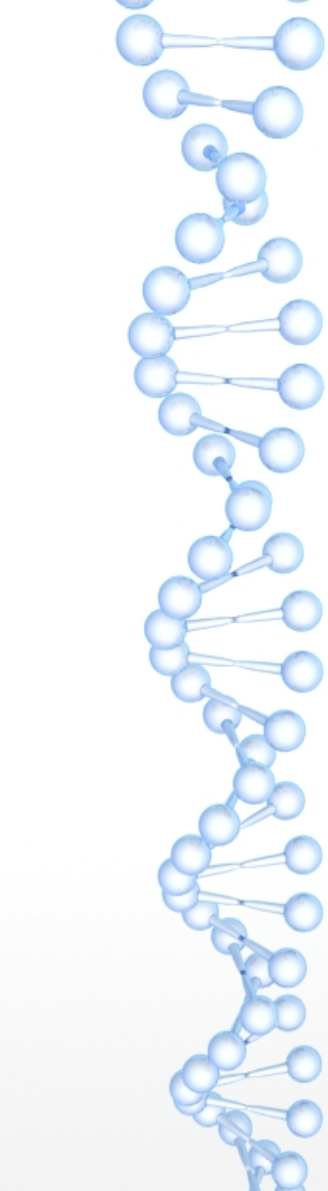
Ang1 promotes the activation of mDia through RhoA, inhibiting SRC-mediated VE-cadherin phospphorylation. In our model RHOA activity is associated with incrreassed permeability, perhaps a diirect interaction from TIE2 to mDia skiping RhoA would be less problematic here.

https://doi.org/10.1016/j.devcel.2007.10.019

Old:
mDia, RHOA
RHOA, !PKGIB & !CDC42 & !ArhGAP29 & !RhoGDIa & (GEFH1 | TIE2 | SRC | G12)
New:
mDia, TIE2
RHOA, !PKGIB & !CDC42 & !ArhGAP29 & !RhoGDIa & (GEFH1 | SRC | G12)

Another option would be:
TIE2 --| RHOA --| mDia
However, see:

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0023448



Oxidative stress

- oxidative_stress, Superoxide | H2O2
- None of the attractors represent cells where oxidative stress is constantly active, this might reflect EC robustness or an error.
- This needs careful analysis, below are the relevant update rules.
- H2O2, SOD
- SOD, Superoxide
- Superoxide, NOX2 | NOX4 | NOX5
- NOX2, O2 & (p47phox | PKC) & (!NRF2 | !propionylLcarnitine)
- NOX4, O2 & HIF1 # This would not activate NOX4 under hypoxic conditions.
- NOX5, O2 & Ca2 & PIP2

- Suggesed new update rules where even under very hypoxic conditions, the basal concentration of oxygen is sufficient to activate the NOX enzymes:
- NOX2, (p47phox | PKC) & (!NRF2 | !propionylLcarnitine)
- NOX4, HIF1
- NOX5, Ca2 & PIP2
- O2 represents normoxia in our model. Hypoxia actually causes oxidative stress(https://ri.conicet.gov.ar/bitstream/handle/11336/40230/CONICET_Digital_Nro.41f41e84-3f10-4ca8-b942-c13b0338f4f3_A.pdf?sequence=2
  see page 19 “under hypoxia, the mitochondrial complex III suffers a conformational change that would facilitate the interaction between O2 and ubisemiquinone, resulting in an increase of O2•− formation (Guzy et al., 2005). Others also consider complex II as relevant for ROS formation during hypoxia exposure (Paddenberg et al., 2003). It was proposed that this complex switches its catalytic activity from succinate dehydrogenase to fumarate reductase at diminished oxygen levels. This would not only cause succinate to accumulate but additionally will cause ROS generation because fumarate reductase has been demonstrated to be a powerful O2•− generator”)
- Also, HIF1 directly activates NOX4 (https://www.molbiolcell.org/doi/10.1091/mbc.e09-12-1003)

Glycocalyx shedding

- glycocalyx_shedding, !Syndecan1
- Syndecan1, heparan_sulfate & !MMP24_25
- heparan_sulfate, heparan_sulfate
- MMP24_25, lysophospholypids & **(Superoxide | H2O2) # We need to examine what happens after the NOX rule change**

Endothelial dysfunction

- **endothelial_dysfunction, !NO | !PGI2**, https://doi.org/10.1113/expphysiol.2007.038588
- For NO see: https://doi.org/10.1161/CIRCULATIONAHA.106.65285, https://doi.org/10.1136/heart.85.3.342, https://doi.org/10.2174/138161282666620051911444 2, https://doi.org/10.1093/cvr/cvq412, Basically NO is a key molecule involved in the modulation of oxidative stress and endothelial barrier permeability.
- For PGI2 see: antiinflamatory https://doi.org/10.3389/fphar.2011.00024, antithrombotic https://doi.org/10.7759/cureus.39967, vasodylator and endothelial barrier function https://doi.org/10.1002/art.41536, its synthesis reduces the concentration of prothrombotic and vasoconstricting prostanoids https://doi.org/10.1161/CIRCRESAHA.124.3249
- In some articles endothelial injury is confused with endothelial dysfunction. EC injury markers include circulating EC microparticles, PECAM1, TM, vWF, TF, ICAM, VCAM, IL6, VEGFA and other molecules: https://www.researchgate.net/profile/Yeon-Ahn/publication/5242087_Endothelial_microparticles_as_markers_of_endothelial_dysfunction/links/563f762c08ae45b5d28d2f4d/Endothelial-microparticles-as-markers-of-endothelial-dysfunction.pdf

Active in all endothelial dysfunction attractors

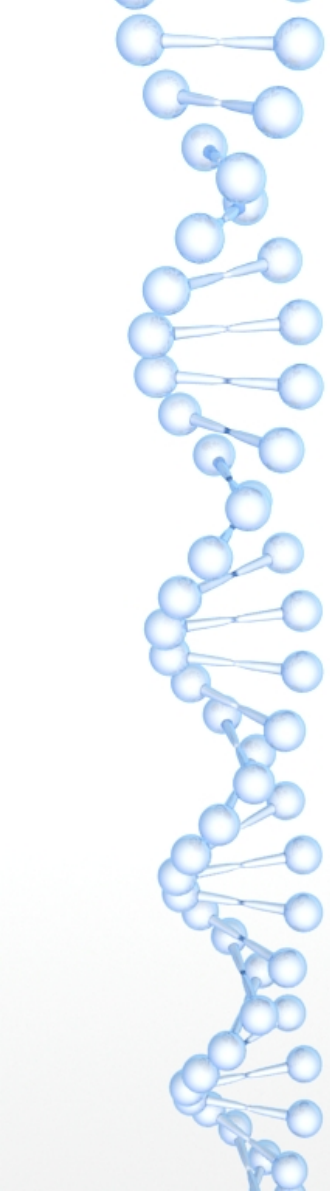
- **AA AC** ADD AF6 AKAP **a_ketoglutarate** ArhGAP29 **cAMP** COX1 CREB3 Cofilin DAG EPAC1 GEFH1 Gq Gs HIF1B **IP** IP3 IP3R KRIT1 lysophospholypids MLCP OXGR1 p120_catenin PAC **PGH2 PGI2** PKA PLC **PPARB** propionylLcarnitine **PTGIS** Phospholamban Phospholypid RAC1 RADIL RAP1 RASIP1 RhoGDIa **TBXAS1** TIAM1 **TP TXA2** VASP VAV2 VHL ZNF185 endothelial_dysfunction
- In all endothelial dysfunction attractors, PGI2 is active, this means that the microenvironmental conditions we used are sufficient for PGI2 and TXA2 production.
- Perhaps cases where **a_ketoglutarate** is inactive are also relevant to fing conditions with insufficient PGI2 production.
- cAMP signaling is also active in all these atractors.

Changes to explore PGI2 synthesis

- **Old: a_ketoglutarate, 1**
- **New: a_ketoglutarate, a_ketoglutarate**

NO deficiency is the only cause of endothelial dysfunction active in our model

- ACTB ANG2 ASFSF2 Bkca CAM cGMP CPI17 Ca2 Calcineurin Cav **eNOS** FAK FOXO1 FilGAP IL6 IL6R IL6ST Integrin IRAG JAK K_hyperpolarisation LCit mDia MEK MLC MLCK NFAT **NO** NOX5 PKC PKGIB RAF RHOA ROCK RRAS sAC sGC SERCA TF TIE1 VEGFR12 VegfR2 vWF
- It is interesting that the molecules associated with NO production are associated with endothelial activation, this just shows how the EC seek homeostasis and quiesceen after activation.



and basic but they will have an important effect on the dynamic behavior of our model that will hopefully take us closer to a functional model. This model is better then I expected for the second draft.