Managing Cancer Stem Cells – the Key to Survival

Neil McKinney, BSc., ND

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- Clinical practice of integrative medicine for 35 + years (retired).
- Cancer Researcher since 1977.
- Founder of BINM and BCNA.
- Professor (retired) and Author in Naturopathic Oncology

Vital Victoria

www.drneilmckinney.ca

Looking over the horizon

- Centuries ago Polynesian voyagers,

expert at intra-island seafaring, set out

in open canoes with plants, pigs, family,

followed currents, wave patterns,

clouds, birds, stars and other natural signs,

to find new islands in the world's largest ocean.

- Stem cell management in oncology is an

emerging field with only pre-clinical

research and clinical experience to guide us.

- Here is where I see we should be heading……

Cancer and Fetal Cells

- Humans start out as an egg and a sperm floating in a fallopian tube. The early embryo creates a mass of relatively undifferentiated cells by symmetric mitosis.
- In time the fetus implants and gets a strong blood supply by building a placenta.
- After the first trimester cells reproduce by asymmetric mitosis. Each stem cell divides into a differentiated cell and a replacement stem cell.
- Cells lost to apoptosis, necrosis, trauma, etc. are replaced by stem cells brought out of dormancy by cell recycling.

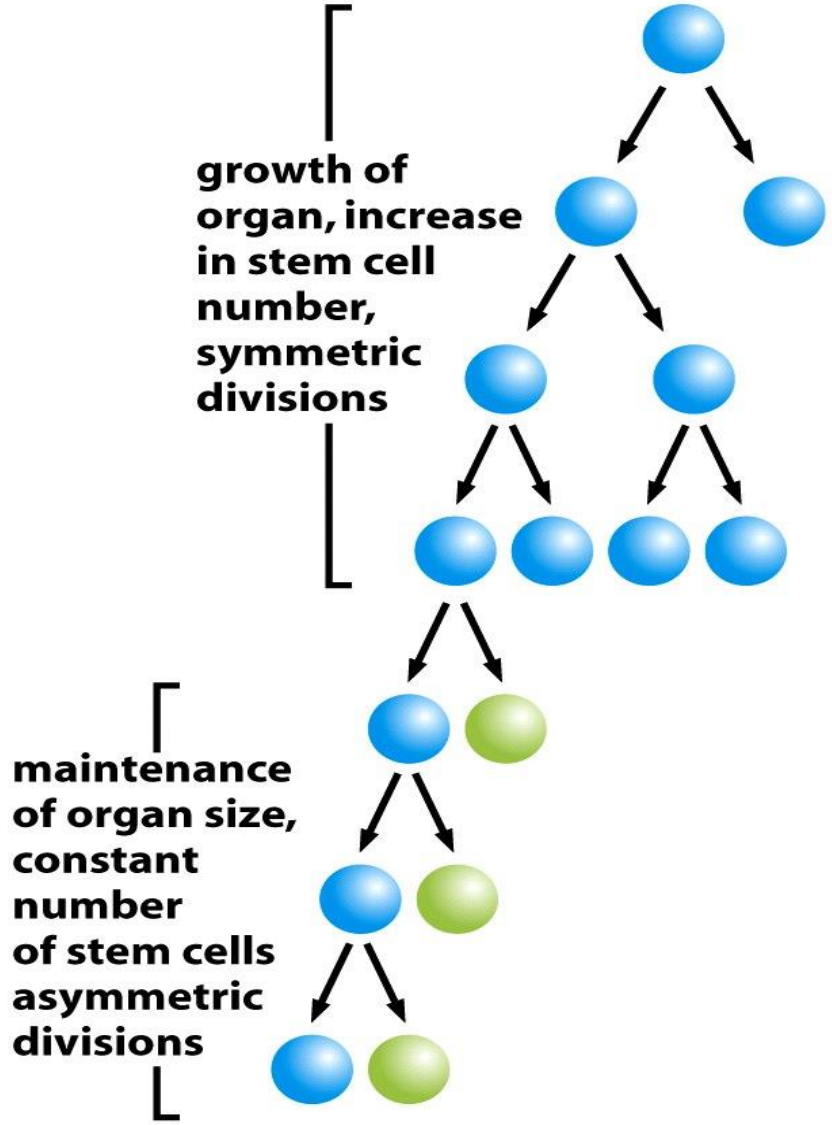


Figure 12-3c The Biology of Cancer (© Garland Science 2007)

Stem cells are critical for tissue repair and longevity.

- Every human tissue has stem cells.
- Stem cells are able to replace any cell that dies of old age, commits suicide because it is damaged or mutated, or is killed by injury, toxicity or disease.
- They can open any part of the genetic code in the chromosomes of the cell, and create a fully differentiated cell specialized to do the job its place in a tissue or organ requires of it. All cells have the DNA library, but most of it is locked down and unusable except very specific parts.
- Stem cells wait quietly for the signal to make a replacement, pull out the blueprints, and make exactly what was lost whole again.

Is Stemness an Adaptation to Hypoxia?

- Fetal cells before implantation and formation of a placenta are relatively *hypoxic, as is tissue with a blood supply damaged by surgery* or trauma.
- Hypoxia impacts normal mitochondrial oxidative phosphorylation. Hexokinase locks them into glycolysis and signals the nucleus to make epigenetic changes in metabolic controls. *Hypoxia favours undifferentiated cell production*.
- *Hypoxia-inducible factor 1* maintains a microenvironment that supports maintenance of stemness.
- Cancer stem cells occupy perivascular niches, as they still require OXYPHOS, but also get ATP via the *Reverse Warburg* effect – recruitment of energy slaves. Cancer cells will die in a completely anoxic environment.

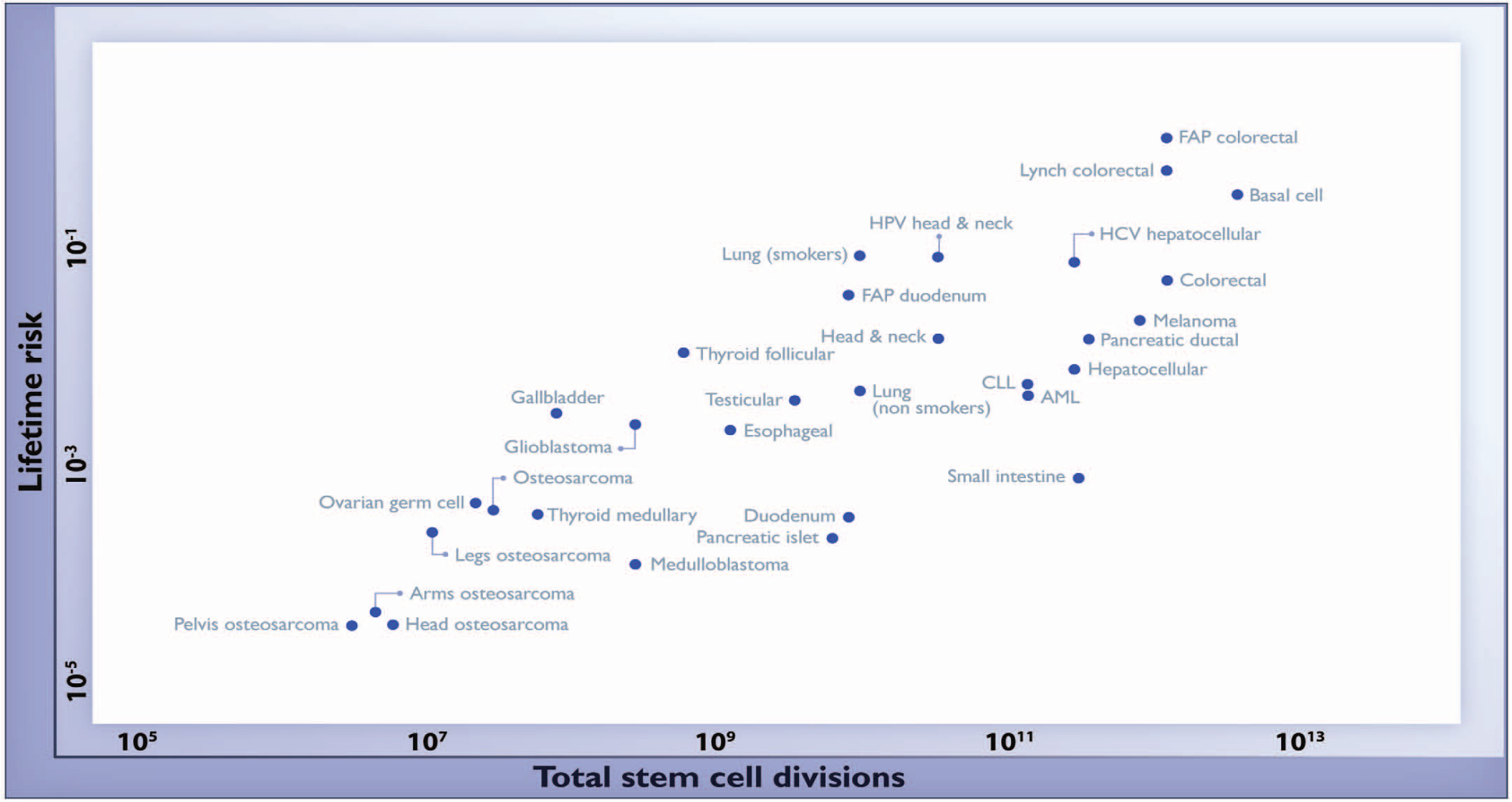
Cancer Stem Cell Theory and the Warburg Effect, Two Sides of the same Coin? Pacini & Borziani, Int. J. Mol. Sci. 2014; 15: 8893-8930.

Interventions for sub-acute hypoxia and hypoxia-inducible factor one HIF1

i. Correct sodium-to-potassium ratio, limit salt intake and increase potassium-rich vegetable and fruit intake.
ii. Alkaline diet -reduce salt, sugar and meat intake.
iii. Aerobic exercise at least twice a week.
iv. Sodium bicarbonate – up to ½ tsp bid.
v. Hyperbaric oxygen HBO2T.

Targeting Hypoxia-Inducible Factor-1, for Cancer Treatment: Recent Advances in Developing Small-Molecule Inhibitors from Natural Compounds, Ma, Xiang, Lee, et al., Semin. Cancer Biol. 2020; https://doi.org/10.1016/j.semcancer.2020.09.011

Stem cell doubling errors are carcinogenic



FAP = Familial Adenomatous Polyposis ◆ HCV = Hepatitis C virus ◆ HPV = Human papillomavirus ◆ CLL = Chronic lymphocytic leukemia ◆ AML = Acute myeloid leukemia

Variation in Cancer Risk Among Tissues Can Be Explained by the Number of Stem Cell Divisions, Tomasetti & Vogelstein, Science 2015; 347 (6217): 78-81.

MYC Oncogene and CSCs

- c-Myc/Max complex inhibits the ectopic differentiation of stem cells.
- Myc-induced epigenetic reprogramming enhances the CSC phenotypes.
- Dormancy of CSCs is due to FBW7-dependent c-Myc degradation pathway.
- Myc-dependent metabolic reprogramming is closely related to CD44 variant-dependent redox stress regulation in CSCs.
- The proto-oncogene c-Myc codes for a nuclear protein transcription factor.
- C-MYC oncogene is inhibited by **berberine, curcumin** and **grapeseed extract** oligomeric proanthocyanidins.

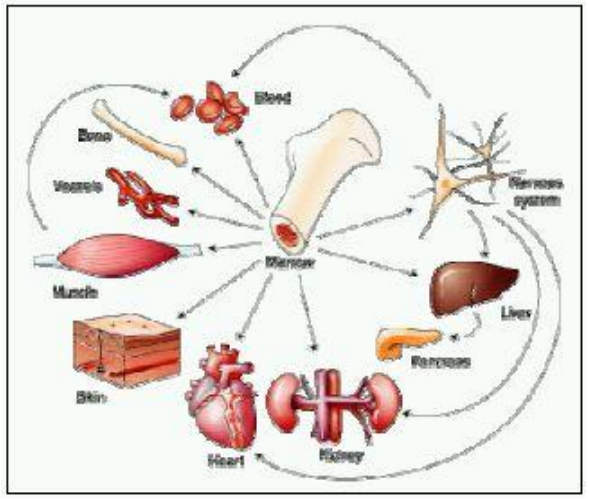
Molecular Mechanisms Behind the Chemopreventive Effects of Anthocyanidins, Hou, Fujii, Terahara & Yoshimoto, Biomed. Biotechnol. 2004; 2004 (5): 321-325.

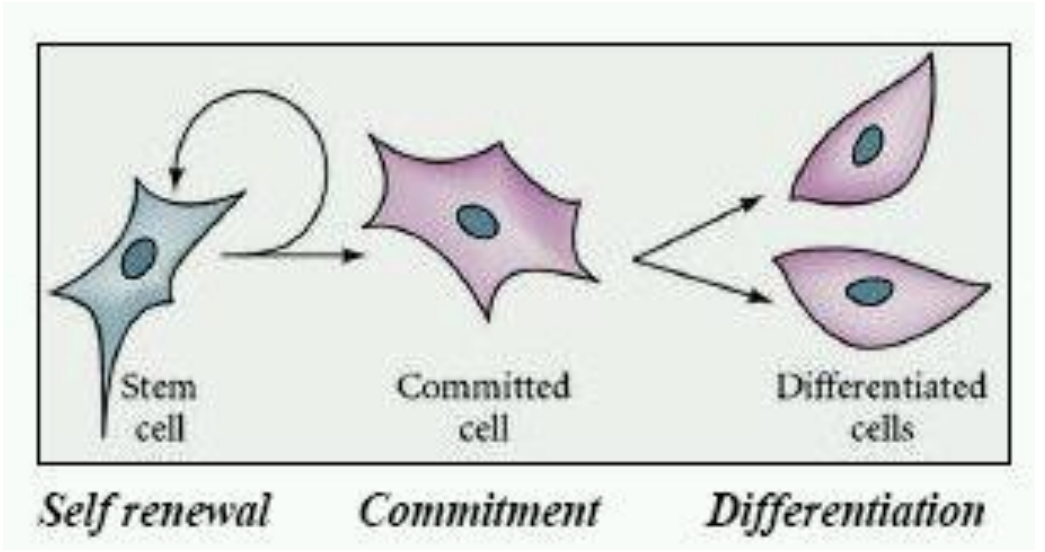
Differential Regulation of p53, c-Myc, Bcl-2 and Bax Protein Expression During Apoptosis Induced by Widely Divergent Stimuli in Human Hepatoblastoma Cells, Jiang, Yang-Yen, Lin & Yen, Oncogene 1996; 13 (3): 609-616.

Emerging Roles of Myc in Stem Cell Biology and Novel Tumor Therapies, Yoshida, J. Exp. Clin. Cancer Res**.** 2018; 37 (1): 173. doi: 10.1186/s13046-018-0835-y

Bone Marrow Stem Cells Support Cancer Cells

- Mesenchymal and **bone-marrow derived stem cells** are recruited to try to manage tumour acidification and inflammation.





- These pluri-potent cells appear to be necessary conditions for the development of the very **malignant properties** of **invasion** and **metastasis**.

Bone Marrow Derived Cells & Cancer – An Opportunity for Improved Therapy, Houghton, Nat. Clin. Pract. Oncol. 2007; 4 (1): 2-3. Medscape article 551187

The Lymphovascular Embolus of Inflammatory Breast Cancer Expresses a Stem Cell-Like Phenotype, Xiao, Ye, Yearsley, et al., Amer. J. Pathol. 2008; 173 (2): 561-574.

Stem cells can become malignant

- Stem cells, under conditions found in tumours, can activate oncogenes and become malignant, ie possess the power of essentially unlimited replicative potential.
- As many as 1 in 4 cells in a melanoma tumour are tumourigenic and stem-cell-like. Perhaps this is why these tumours spread early and aggressively.
- These **cancerized stem cells** are trained to produce chemicals that maintain the tumour. The abnormal cell-to-cell contacts in the tumour and the wash of growth factors convert the stem cells into malignant cells. The stem cells make actually fuse into some cancer cells, making a hybrid with the power to grow wildly. They are then able to make new full-blown tumour cells.

Cancer Stem Cells: The Promise and the Potential, Ajani, Song, Hochster, et al., Semin. Oncol. 2015; 42 (Suppl. 1): S3-S17.

Will Cancer Stem Cell Provide New Therapeutic Targets?- Review, Behbod & Rosen, Carcinogenesis 2004; 26 (4): 703-711.

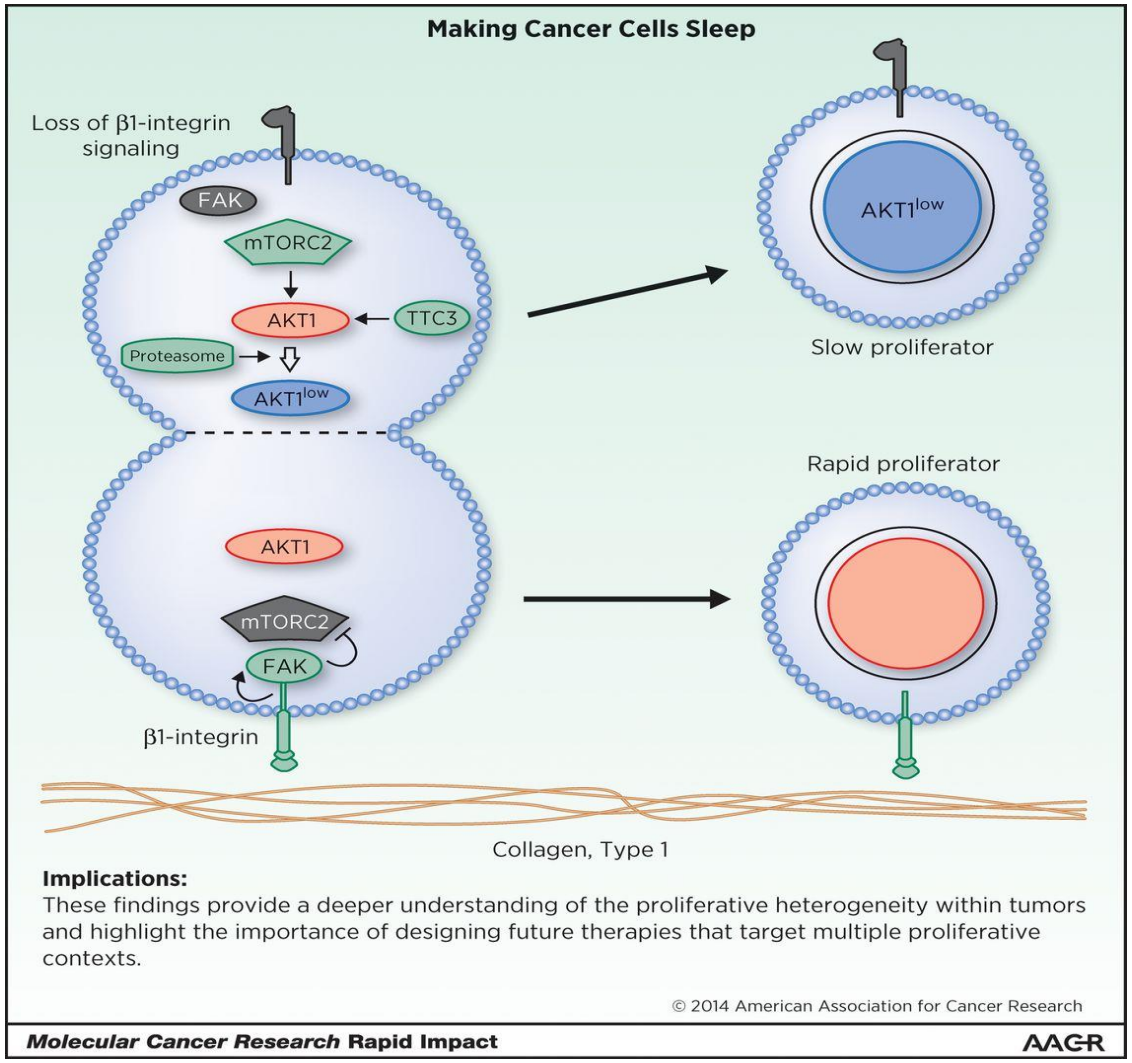
Malignant cells can become stem cells

- Tumours actively recruit mesenchymal stem/stromal cells. Cancer cells under stress can **cannibalize** these stem cells and gain the ability to become dormant.
- Asymmetrical mitosis of cancer cells can produce cells deficient in **akt**, a protein kinase integral to cell division. “Decreased signaling through the cell-surface molecule **beta 1-integrin** decreases activity of the signaling molecule FAK (also known as protein tyrosine kinase 2 [**PTK**2]). The reduced FAK signaling leads to increased activity of the **mTOR**C2 protein complex, which in turn leads to suppression of AKT1 levels because of a one-two hit from TTC3, a ubiquitin protein, and proteasome complex, a cellular housekeeping service”.

*A Mechanism for Asymmetric Cell Division Resulting in Proliferative Asynchronicity***,** Dey-Guha, Alves, Yeh, et al., Mol. Cancer Res. 2015; published online January 12, 2015.

Cancer Cells Enter Dormancy After Cannabilizing Mesenchymal Stem/Stromal Cells (MSCs), Bartosh, Ullah, Zeitouni, et al., PNAS 2016, E6447-E6456. Oct. 3, 2016 doi/10.1073/pnas.1612290113.

“Slow” cancer cells resist therapy

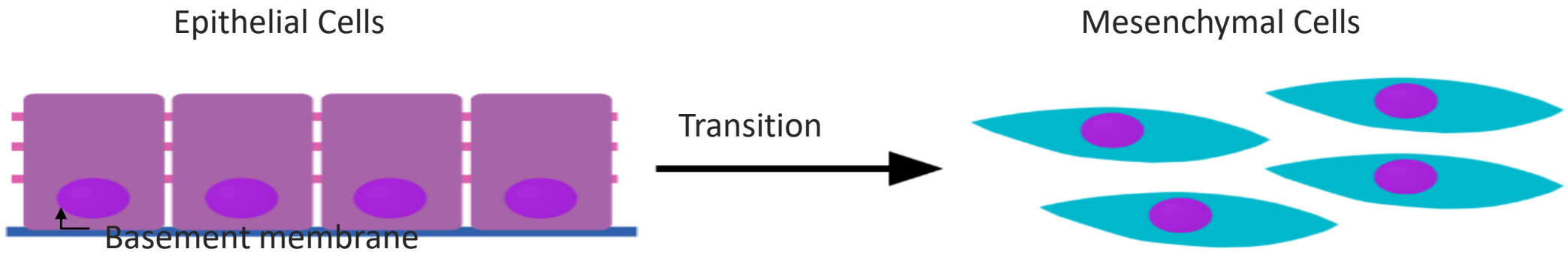


The Cancer Sleeper Cell, Siddhartha Mukherjee, 2010, adapted from “Emperor of All Maladies: A Biography of Cancer”.
Cancer Stem Cell – Perspectives On Current Status and Future Directions, Clarke, Cancer Res. 2006; 66 (19): 9339-9344.

Interventions to inhibit akt/mTOR pathway

i. Curcumin
ii. Green tea EGCG
iii. Indole-3-carbinol
iv. Metformin.

The epithelial mesenchymal transition



- The epithelial to mesenchymal transition is an essential part of wound healing and embryogenesis. It can be triggered by cancer surgery or cytotoxic therapy.
- EMT cells lose adhesion eg e-cadherin (CDH1), acquire motility, and can float freely into lymphatics and blood vessels.
- Hypoxia, nutrient deprivation, micro RNA and mutations may trigger this transition.
- Transforming growth factor-β (TGF-β) is an inducer of the epithelial-mesenchymal transition in epithelial cells and obligatory for an invasive phenotype in carcinoma.
- Citrate synthase deregulation promotes the epithelial-mesenchymal transition. So does the autophagy defensive response.

Epithelial-Mesenchymal Transition and the Stem Cell Phenotype, Radinsky & LaBarge, Cell Stem Cell 2008; 2(6): 511-512.

The Systemic Response to Surgery Triggers the Outgrowth of Distant Immune-ControlledTumors in Mouse Models of Dormancy, Krall, Reinhardt, Mercury, et. al., Sci. Transl. Med. 2018; 10: eaan3464.

Plasticity of CSCs and EMT

The plasticity of cancerized stem cells (CSCs) allows the cells to transition between epithelial and mesenchymal states, which is what gives them the ability to invade tissue, disseminate, and grow at metastatic sites.

Qualities of Epithelial-like vs CSC Mesenchymal-like State

Epithelial-like State	**Mesenchymal-like State**
• Express epithelial markers • Have polarity • Proliferate extensively • Found more in tumor interior	• Express mesenchymal markers • Remain relatively quiescent • Found at tumor invasive front • Invade the bloodstream • Establish micrometastases

Breast Cancer Stem Cells Transition Between Epithelial and Mesenchymal States Reflective of Their Normal Counterparts, Liu, Cong, Wang, et al. Stem Cell Reports 2013; 2 (1): 78-91.

Interventions for the Epithelial-Mesenchymal Transition

i. CBD cannabidiol
ii. Curcumin
ii. Berberine
iii. Resveratrol
iv. Green tea EGCG
v. PPARγ agonists

Expression and Function of PPARs in Cancer Stem Cells, Zhang, Zhang, Wang, et al., Curr. Stem Cell Res. Ther. 2016; 11 (3): 226-234.

Cannabidiol (CBD) in Cancer Care, McKinney, Can. J. Naturopathic Med., Dec. 2021; 1 (3): 7-22.

CBD Reverts the Mesenchymal Invasive Phenotype of Breast Cancer Cells Induced by the Inflammatory Cytokine IL-1, García-Morales, Castillo, Ramírez, et al., Int. J. Mol. Sci. 2020; 21: 2429.

Metformin Inhibits the Inflammatory Response Associated with Cellular Transformation and Cancer Stem Cell Growth, Hirsch, Iliopoulos& Struhl, PNAS 2013: 110 (3): 972-977.

CXCR-4 G-protein coupled receptor

- The chemokine CXCL12, also known as stromal cell-derived factor-1, also known as “fusin,”, binds the G-protein-coupled receptor CXCR4, which, through multiple divergent pathways, leads to chemotaxis, enhanced intracellular calcium, cell adhesion, survival, proliferation, and gene transcription.
- Cancer cell CXCR4 overexpression contributes to tumor growth, invasion, angiogenesis, metastasis, relapse, and therapeutic resistance.
- The CXCR4/CXCL12 axis is indispensable for cell migration during embryonic hematopoiesis, organogenesis, vascularization, and organ homeostasis = linked to embryonic cell behaviour.

Diversity and Inter-Connections in the CXCR4 Chemokine Receptor/Ligand Family: Molecular Perspectives, Pawig, Klasen, Weber, et al., Front. Immunol. 2015, 6: 429.

Involvement of CXCR4 in Normal and Abnormal Development, Kawaguchi, Zhang & Nakanishi, Cells 2019; 8: 185.

CXCL12 (SDF1alpha)-CXCR4/CXCR7 Pathway Inhibition: An Emerging Sensitizer for Anticancer Therapies?, Duda, Kozin, Kirkpatrick, et al., Clin. Cancer Res. 2011; 17 (8): 207420-80

- The CXCR4/CXCL12 axis plays a critical role in therapeutic resistance by

(i) directly promoting cancer cell survival, invasion, and cancer stem (or tumor-initiating) cell phenotype.

(ii) recruiting myeloid bone marrow-derived cells to indirectly facilitate tumor recurrence and metastasis.

(iii) promoting angiogenesis directly or in a paracrine manner CXCR4 signaling via AKT leads to stabilization of β-catenin. Stabilized β-catenin moves to the nucleus and activates gene transcription and promotes proliferation.

- CXCR4 antagonism has been shown to disrupt tumor–stromal interactions, sensitize cancer cells to cytotoxic drugs, and reduce tumor growth and metastatic burden.

An Infernal Trio: The Chemokine CXCL2and its Receptors CXCR4 and CXCR7 in Tumor Biology, Hatterman & Mentlein, Ann. Anat. 2013; 195 (2): 103 -10.

Targeting CXCL12/CXCR4 Axis in Tumor Immunotherapy, Zhou, Guo, Liu, et al., Curr. Med. Chem. 2019; 26 (17): 3026–3041.

Involvement of CXCR4 in Normal and Abnormal Development, Kawaguchi, Zhang & Nakanishi, Cells 2019; 8: 185.

Interventions for CXCR-4 G-Protein Receptor

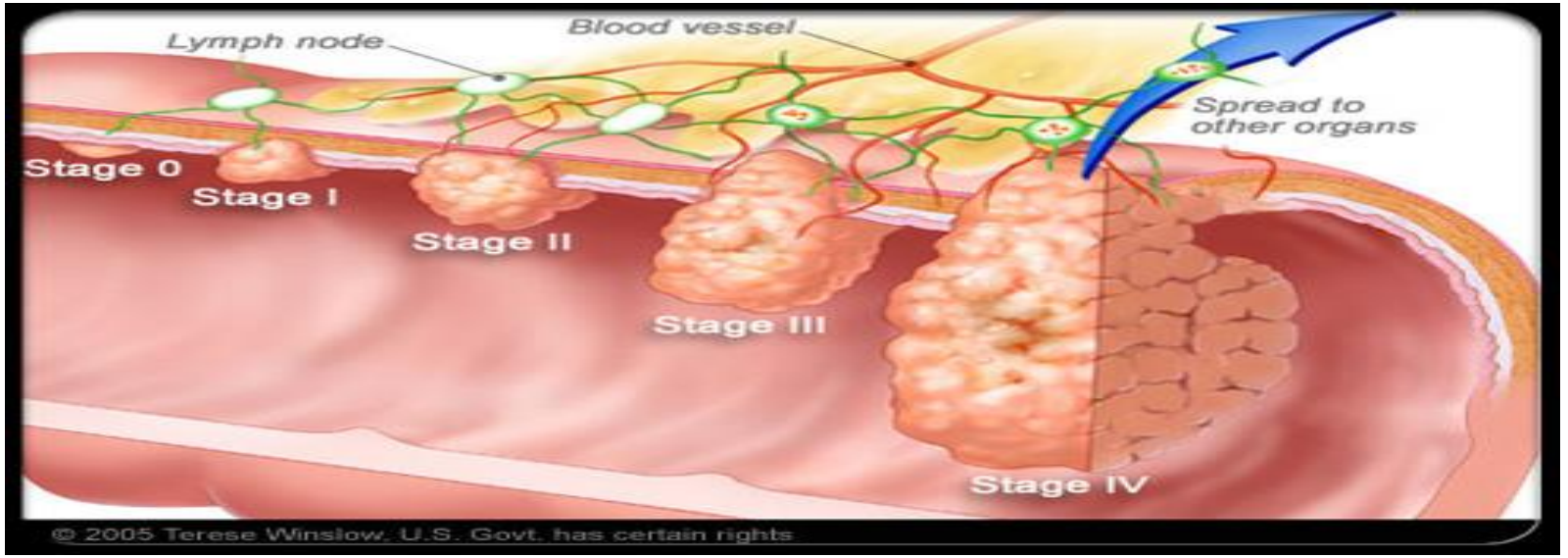
i. Boswellia
i. Quercetin
ii. Reishi extract
iii. Cannabidiol (CBD)
iv. Curcumin
v. Thymoquinone from blackseed *Nigella sativa*

Interventions for CXCL12 fusion chemokine

i. Tannic acid
ii. Quercetin

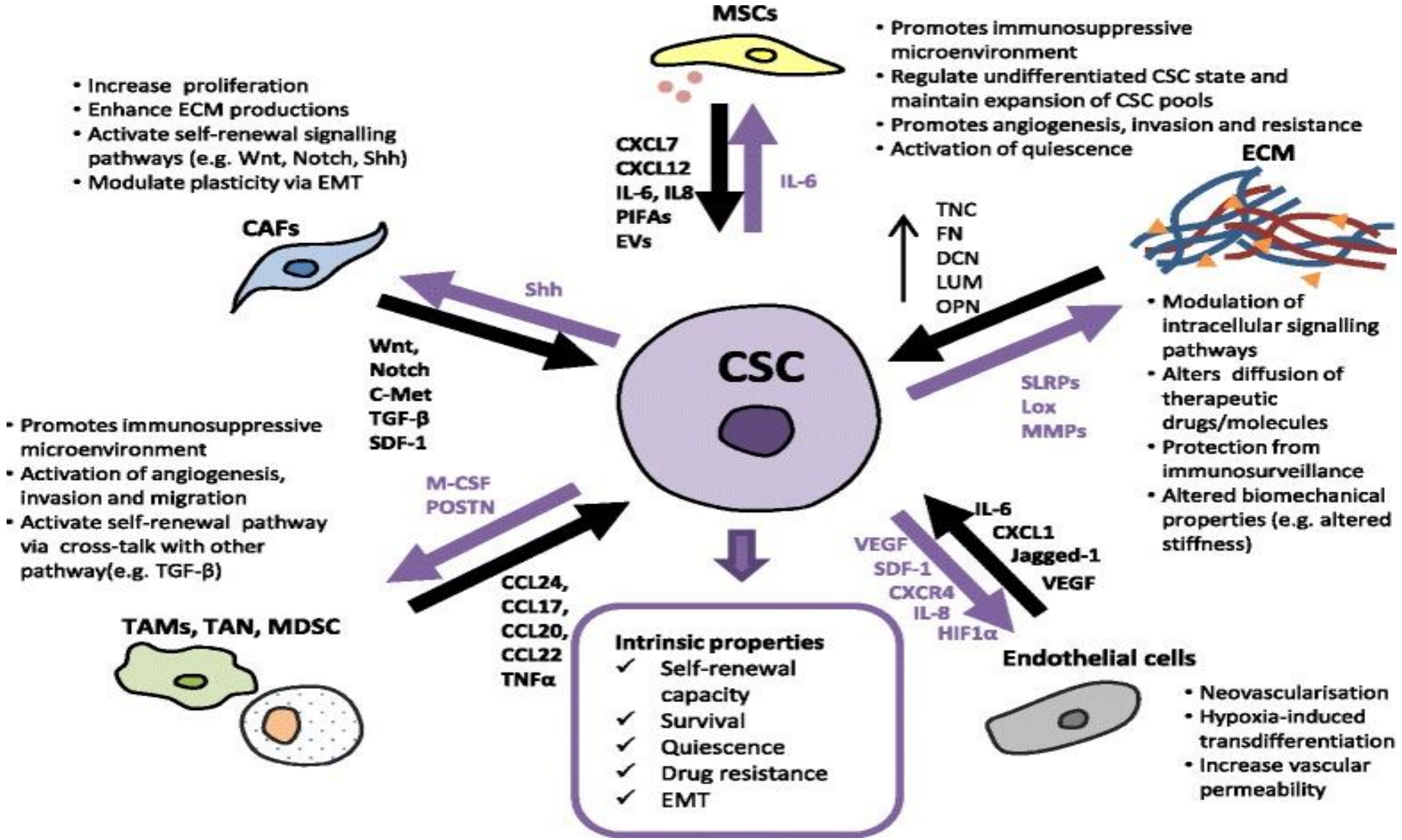
Stem cells can do bad things

- Only a minority of tumour cells can initiate a tumour.
- Pluripotent stem cells are native to the human breast and other tissues.
- **Cells with "stemness" can invade and metastasize**. They can divide without being anchored.



Cancer Stem Cells: Are We Missing the Target? – Commentary, Jones, Matsui & Smith, J. Natl. Cancer Inst**.** 2004; 96 (8): 583-585.

Cancer Stem Cells with Increased Metastatic Potential as a Therapeutic Target for Esophageal Cancer, Wang, Plukker & Coppes, Sem. Cancer Biol. 2017; 44: 60-66.



Cancer Stem Cells As Key Drivers of Tumour Progression, Ayob & Ramasany, *J. Biomed. Sci*. 2018; 25 (1): 20.

Dormancy hides cancer from oxidative therapies

- Tumours resist chemotherapy via the P-glycoprotein porter system, and there are always drug delivery issues into hypoxic areas.
- Oxygen transduces photonic energy into chemical damage at a molecular and organelle level in radiation therapy. Hypoxia = radio-resistance.
- Radio-resistance and chemo-resistance are existing properties of non-cycling cells.
- These resistant properties are shared by low-akt sleeper cells.

Cancer Stem Cells, Cancer Cell Plasticity, and Radiation Therapy, Vlashia & Pajonka, Semin. Cancer Biol. 2014; 28-35.

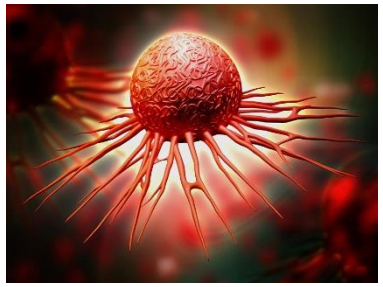
Tumour Stem Cells: Rooting Out Resistance, Rich, et al., Nat. Rev. Cancer 2006; 6 (12): 904-905. Medscape article 550020

The Paradox of Response and Survival in Cancer Therapeutics, Huff, Matsui, Smith & Jones, Blood 2006; 107: 431-434.

Chemoresistant 'Sleeper' Cancer Cells May Be Targetable, Ramaswamy, Mol. Cancer Res. 2015;

Late reoccurrences in breast cancer

- Cancerized stem cells can be found in the bone marrow of breast cancer patients for decades after diagnosis.
- These can reactivate and cause the recurrence of genetically identical cancers up to 20 years after an apparent cure.
- Beyond 2 years from curative therapy, this may be a leading cause of recurrence.



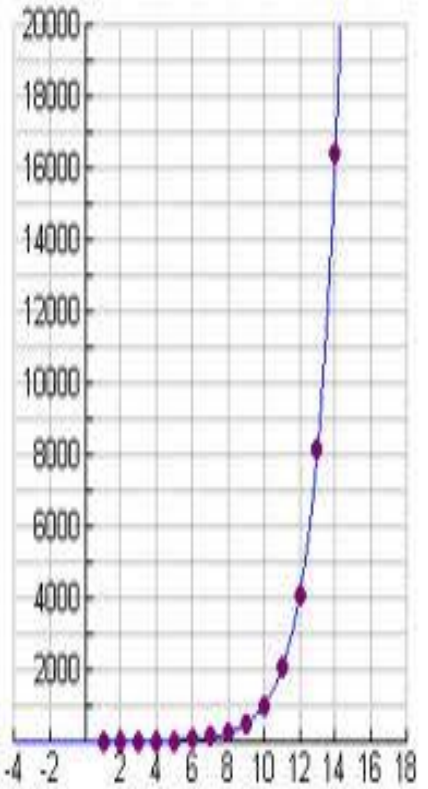
Insights into Breast Cancer Heterogenicity – Stem Cells, Skinner, Nat. Rev. Cancer 2010; 10 (3): 163.

On Mammary Stem Cells, Woodward, Chen, Behbod & Rosen, J. Cell Sci. 2005; 118: 3585-3594.

ER Alpha Status of Disseminated Tumour Cells in Bone Marrow of Primary Breast Cancer Patients, Fehm, Krawczyk, Solomayer, et al., Breast Cancer Res. 2008; 0 (5): R76.

CSCs and Progenitors

- Cancer stem cells can give rise to sterile tumour cells and more CSCs, by asymmetrical division.
- CSCs can differentiate into *progenitor cells capable of symmetrical mitosis, to the Hayflick limit*. Progenitors can mutate or be altered by epigenetic influences into various hierarchical cell lines, thus explaining the great heterogeneity of cells in tumours.
- Progenitor cells can mutate into *CSCs with unlimited renewal capacity.*
- Ordinary mature tumour cells can also mutate or transition into de-differentiated cancerized stem-cells or stem-cell-like cells capable of unlimited division.



A Mechanism for Asymmetric Cell Division Resulting in Proliferative Asynchronicity, Dey-Guha, Alves, Yeh, et al., Mol. Cancer Res. 2015; published online January12, 2015.

Heterogeneity in Cancer: Cancer Stem Cells Versus Clonal Evolution, Shackleton, Fearon, Quintana & Morrison, Cell 2009; 138 (5):822-829.

Cancer Progenitors and TGF-β

- Genes characteristic of neural and other stem cells are expressed in brain tumour-derived progenitors (TDP): CD 133, Sox-2, musahi-1, bmi-1, phosphoserine phosphatase, maternal embryonic leucine zipper kinase.
- TDPs are multi-potent, capable of self-renewal and differentiation into abnormal phenotypes.
- To become fully cancerized they must escape the anti-proliferative effect of the neuro-protectant TGF-beta. This multi-functional cytokine mediates inflammation and immune escape mechanisms.

Brain Tumour Stem Cells, Vescovi, Galli, and Reynolds, Nat. Rev. Cancer 2006; 6 (1): 425-436.

Cancerous Stem Cells Arise from Pediatric Brain Tumours, Nakano, Lazareff, et al., PNAS 2003; 100 (25): 15178-15183.

Interventions for Transforming Growth Factor Beta

i. R-alpha lipoic acid
ii. Quercetin
iii. Berberine
iv. Curcumin
v. Metformin
vi. Green tea EGCG

Other TGF-β Regulators

To control stemness, angiogenesis, metastasis, immune suppression and oxidative stress:

- Taurine, lycopene, licorice root, pokeroot, silymarin omega 3 fish oils, vitamin C, resveratrol, ginkgo biloba, rehmannia.
- Reduce obesity to restrict leptins linked to TGFβ-1 signaling.
- IV-vitamin C with Doxycycline?

Vitamin C and Doxycycline: A Synthetic Lethal Combination Targeting Metabolic Flexibility in Cancer Stem Cells (CSCs), De Francesco, Bonucelli, Maggliolini, et al., Oncotarget 2017; 8 (40): 67269-67286.

Targeting Cancer Stem-Like Cells Using Dietary-Derived Agents – Where Are We Now?, Khan, Kamokar, Howell, et al., Mol. Nutr. Food Res. 2016; 60 (6): 1295-1309.

TGF-beta in Neural Stem Cells and in Tumors of the Central Nervous System, Aigner & Bogdahn, Cell Tissue Res. 2008: 331 (1): 225-241.

Peroxisome Proliferators-Activated Receptor Gamma

- Peroxisome proliferators-activated receptor gamma - PPARγ - is involved in tumor growth and invasiveness, interacting with beta-catenin.
- PPARγ agonists deplete stem cell pools.
- PPARϒ modulates the epithelial-mesenchymal transition.
- PPARϒ agonists inhibit mesenchymal stem cells (Zhang, 2016; Rousseau, 2018; Yousefnia, 2018).

The Influence of Peroxisome Proliferator-Activated Receptor ϒ(PPARϒ) Ligands on Cancer Cell Tumourigenicity, Yousefinia, Momendzadeh, Forootan,et al., Gene 2018; 649:14-22.

Expression and Function of PPARs in Cancer Stem Cells, Zhang, Zhang, Wang, et al Curr. Stem Cell Res. Ther. 2016; 11 (3): 226-234.

Natural Interventions for Peroxisome Proliferator-Activated Receptor Gamma (PPARγ)

- Fermented wheat germ extract (FWGE) 9 gm qd –bid
- R-alpha lipoic acid
- Red wine 1 glass daily.
- Omega 3 oil DHA
- Coffee
- Niacin
- Δ9-THCA
- CBD
- DHA and EPA omega 3s
- PEA – palmitoylethanolamide

α-Lipoic Acid Up-Regulates Expression of Peroxisome Proliferator-Activated Receptor β in Skeletal Muscle: Involvement of the JNK Signaling Pathway, Rousseau, Sibille, Murdaca, et al., FASEB J. 2016; (3):1287-1299.

Palmitoylethanolamide (PEA): Clinical Applications, McKinney, Can. J. Naturopathic Med., June 2021;1 (1): 55-66.

Interventions to Control Beta-Catenin

i. Quercetin
ii. Berberine
iii. Curcumin
iv. Metformin
v. Vit. A retinol
vi. Indole-3-carbinol/DIM, sulforaphane

Interventions for Wtn/β-catenin stem cell signaling pathways Hedgehog, Notch Wnt & Bmi.

- Quercetin
- Piperine
- Green tea EGCG
- Sulforaphane
- Genestein
- Curcumin
- Vitamin D3
- Berberine
- Resveratrol
- Beta-carotene

Phytochemicals as Innovative Therapeutic Tools against Cancer Stem Cells, Scarpa & Ninfali Int. J. Molec. Sci. 2015; 16 (7): 15727-15742.

Other targets in managing stemness

- **inhibit NFκB,** the critical regulator of growth promoting genes in stem cells.
- **promote PTEN** tumour suppressor gene activity.
- **block the ABC transporters** such as the P-glycoprotein porter.
- **block IL-8 and IL-6** inflammatory mediators released by dying cells → CSC survivors.
- **force differentiation**, restoring sensitivity to cytotoxic agents.
- **anti-angiogenesis** targets malignant bone marrow-derived stem cells.
- **JAK/STAT pathways** including STAT3.

Cancer Stem Cells: The Promise and the Potential, Ajani, Song, Hochster, et al. Semin. Oncol**.** 2015; 42 (Suppl. 1): S3-S17.

Targeting NFκB for Cancer Cell Stemness:

- Nuclear factor NFκB is a critical regulator of growth-promoting genes in stem cells (Torquato, 2017).
- Interventions: curcumin, quercetin, proanthocyanidins, reishi, black seed *Nigella sativa* thymoquinone, green tea EGCG, metformin, indole-3-carbinol, DIM, R-alpha lipoic acid, ginger, silibinin, feverfew parthenolides, selenium, zinc, vitamin C, vitamin D, vitamin K3, calcium, gamma vitamin E, beta carotene, apigenin, melatonin, resveratrol, emodin, genestein, guggulsterone, zerumbone, evodiamine, aspirin, salicylic acid, holy basil ursolic acid, melanin (echinacea, black cumin, tea), ginkgo biloba, niclosamide.

Targeting Differentiation

- Forcing differentiation may reduce *stemness*.
- Bone morphogenetic proteins (BMPs) esp. BMP4 induce differentiation in glioblastoma cancer stem cells.
- Interventions for differentiation –**berberine, quercetin, Vit. C**, and perhaps boswellia, butyrate, bromelain, vitamin D, LAMC, melatonin, burdock root, retinoids, vitamin A.

  eg **Retinol vitamin A** 25,000 IU daily; (30K palmitate), if AST > 300 – 5,000 IU/d.

Bone Morphogenetic Proteins Inhibit the Tumorigenic Potential of Human Brain Tumour-Initiating Cells, Piccirillo & Vescovi, Nature 2006; 444: 761-765.

Role of Transglutaminase 2 in Quercitin-Induced Differentiation of B16-F10 Murine Melanoma Cells, Forni, Braglia, Lentini, Nuccetelli, Amino Acids 2008; 4: 731-738.

Metabolic Therapies in Advanced Cancer Salvage Cases, Paul Anderson, Townsend Letter Aug/Sept. 2018; p. 39-43.

- **promote PTEN** tumor suppressor gene activity - interventions: honokiol, indole-3-carbinol, diindolymethane, curcumin, green tea, quercetin, genestein, isoflavones, butyrate, omega 3 DHA, resveratrol, astragalus,rhodiola, thymoquinone, ashwagandha, sulforaphane, licorice root.

- **block the ABC transporters** such as the P-glycoprotein porter – intervention: quercetin.

- **Anti-angiogenesis** -targets malignant bone marrow-derived stem cells (Teicher, 2011), interventions: mistletoe, catechin, curcumin, green tea EGCG, quercetin, grapeseed anthocyanidins, pomegranate, resveratrol, sea cucumber extract, beta carotene, coriolus PSK, genestein, EPA oils, shark liver oil, selenium, zinc, luteolin, lysine, proline, vitamins A, C, D & E, modified citrus pectin, milk thistle, bupleurum, sanguinaria, rabdosia, ginseng, wormwood, scutellaria, honokiol (magnolia), poria, ginkgo, angelica, polygonum, COX-2 inhibitors.
- **Inhibit JAK/STAT pathways** including STAT3 (Liu, 2018), interventions: indole-3-carbinol, DIM (which also suppress MSCT, differentiation), curcumin, doxycycline, curcubitacin Q, niclosamide.

Modulation of the Constitutive Activated STAT3 Transcription Factor in Pancreatic Cancer Prevention: Effects of Indole-3-Carbinol (I3C) and Genestein, Lian, Word, Taylor, et al., Anticancer Res. 2004; 24 (1): 133-137.
Novel STAT3 Phosphorylation Inhibitors [from Curcumin Exhibit Potent Growth-Suppressive Activity in Pancreatic and Breast Cancer Cells, Lin, Hutzen,Zuo, et al., Cancer Res. 2010; 70 (6): 2445-2454. .
STAT3/p53 Pathway Activation Disrupts IFN-β-Induced Dormancy in Tumor-Repopulating Cells, Liu, Lv, Liu, et al., J. Clin. Invest. 2018; 128(3):1057-1073.
Naturopathic Oncology-An Encyclopedic Guide for Patients and Physicians, 4th ed., McKinney, 2020, Liaison Press.

- **block IL-8 and IL-6** inflammatory mediators released by dying cells → CSC survivors; IL-6 is also called B-cell stimulatory factor BSF-2, raises CRP, and is produced by peripheral lymphocytes and monocytes; IL-8 is an attractant of neutrophils, goes up in tumour fever; interventions: black seed *Nigella sativa* thymoquinone, green tea EGCG, melatonin, R-ALA, mushroom extracts, vit C, resveratrol, vit D3, *Sophora flavescens* root oxymatrine and matrine, hesperidin methyl chalcone.

- **Support p53** DNA protector and repair - interventions: quercetin, curcumin, green tea EGCG, grapeseed OPC's, retinoic acid, genestein, selenomethionine, melatonin, catechin, garlic, vit. C trans-resveratrol, gamma vitamin E, folate, milk thistle, N-acetyl-cysteine.

Insulin and IGF-1 and breast cancer stem cells

- Breast cancer cells have extra insulin receptors, and estrogen receptors are amplified by IGF-1. These also drive the EMT.
- Natural interventions that impact this nutrition sensitive system: exercise, caloric restriction, ketogenic diet, methionine restriction, berberine, vanadium, green tea EGCG, R-alpha lipoic acid, I3C/DIM.

Insulin and Cancer, Boyd, Integr. Cancer Ther., 2003; 2 (4): 315-329.

Epithelial-to-Mesenchymal Transition in Breast Cancer: A Role For Insulin-Like Growth Factor I and Insulin-Like Growth Factor–Binding Protein 3?, Zielinska, Bahl, Holly & Perks, Breast Cancer: Targ. Ther. 2015;7: 9–19.

Surgery, Inflammation, Wound Healing & Stem Cells

- Surgery, wounding, trauma and inflammation bring localized stem cells out of dormancy, to regenerate cells and tissues. If inflammation becomes extreme, bone-marrow derived stem cells are recruited, and new stem cells arise via the epithelial mesenchymal transition.
- Cancer cells are at increased risk of stemness acquisition in the immediate aftermath of surgery, or inflammation from other causes including radiation therapy.
- Outcomes are improved by addressing surgical immunosuppression, addressing inflammation, limiting opiates, and managing stem cell formation.

The Effect of Paravertebral Block During Surgery on the Recurrence of Breast Cancer, Oppfeldt, Palle & Carlsson, Amer. Soc. Anaesth. 2013, Oct. 15, abstract 4253.

Wounding from Biopsy and Breast Cancer Progression, Retsky, Demichelli & Hrushesky, Lancet 2001; 357 (9261): 1048.

The Systemic Response to Surgery Triggers the Outgrowth of Distant Immune-Controlled Tumors in Mouse Models of Dormancy, Krall, Reinhardt, Mercury, et. al., Sci. Transl. Med. 2018; 10: eaan3464.

Interventions for post-op inflammation

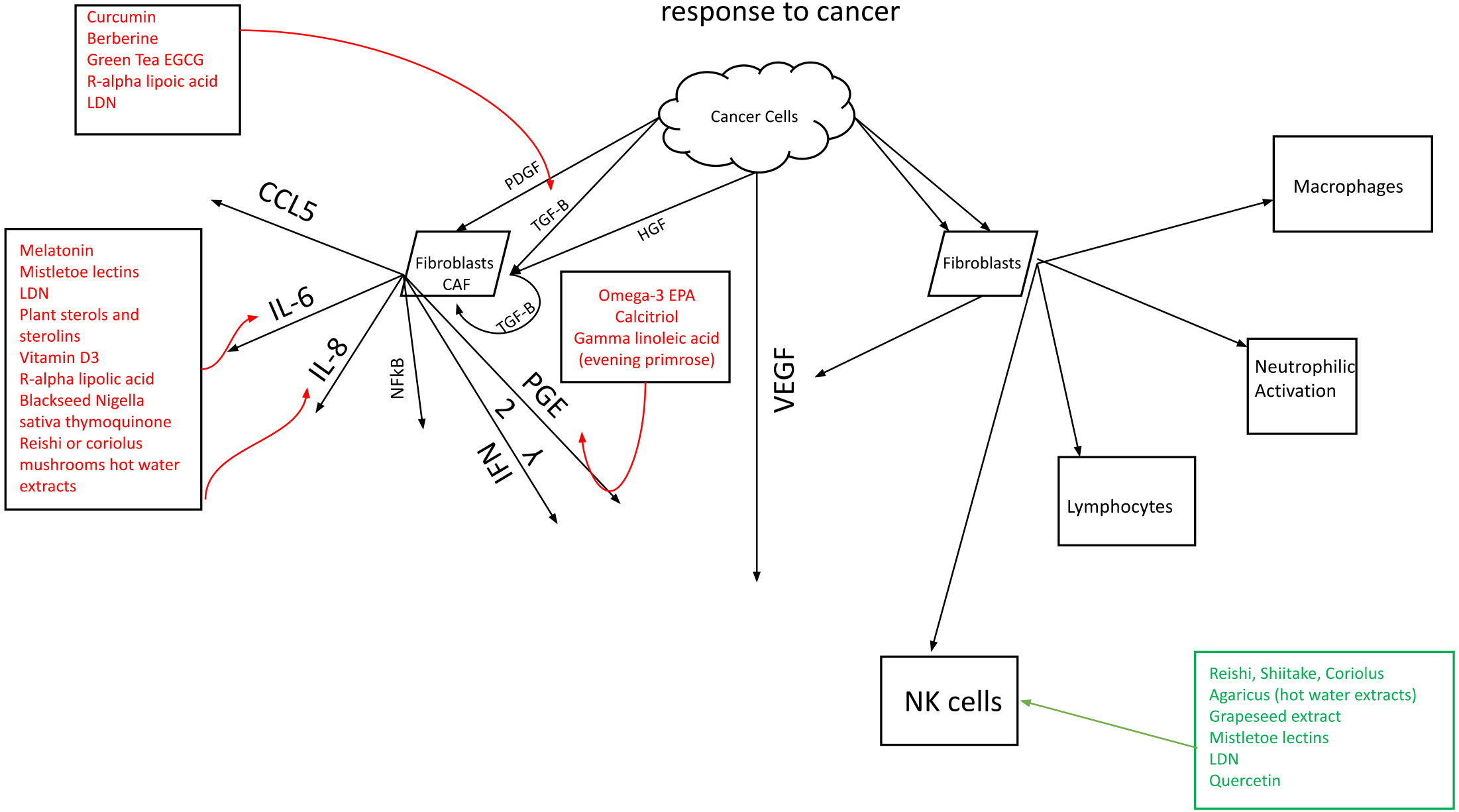
i. Boswellia
ii. Curcumin
iii. Berberine
iv. Grapeseed extract
v. Green tea polyphenols and catechin

Tumour Dormancy and Surgery-Driven Interruption of Dormancy in Breast Cancer: Learning from Failures, Demicheli, Retsky, Hrushky & Baum, Nat. Clin. Pract. Oncol. 2007; 4 (12): 699-710. Review.

The Effects of Surgery on Tumour Growth: A Century of Investigations, Demicheli, Retsky, Hrushky, et al., Ann. Oncol. 2008; 19 (11): 1821-1828.

Surgical Stress and Cancer Progression: The Twisted Tango, Chen, Zhang, Xu, et al., Molec. Cancer 2019; 18: 132.

Inhibit the Inflammatory "wound-healing" response to cancer



Avoid Opiates

- Morphine promotes stemness, metastatsis and chemo-resistance.
- Opiates promote the Wnt/beta-catenin signaling pathway, inducing epithelial to mesenchymal transition.
- Overcome by Nalmefene, a morphine antagonist.
- So perhaps Rx Low-dose Naltrexone? CBD?
- General anaesthesia may also increase cancer recurrence

MIlian, Mata, Alcacer, et al., *Cannabinoid Receptor Expression in Non-Small Cell Lung Cancer. Effectiveness of Tetrahydrocannabinol and Cannabidiol Inhibiting Cell Proliferation and Epithelial- Mesenchymal Transition* in vitro, PLosONE 2020; 15 (2): e0228909.

Recurrence of Breast Cancer After Regional or General Anaesthesia: A Randomised Controlled Trial, Sessler, Pei, Huang, et al., Lancet 2019 Oct 18;[EPub Ahead of Print].

Morphine Promotes Cancer Stem Cell Properties, Contributing to Chemo-Resistance in Breast Cancer, Niu, Peng, Zhang, et al., Oncotarget 2015; 6 (6): 3963-3976.

CSC Markers and Pathways

- myeloid leukemia cells that express a specific surface marker CD34+, but lack the CD38- marker.
- Stem-like CD44+/CD24- tumour cells can be isolated from human breast cancer tissue and many other cancers
- Stemness also commonly associates with increased ALDH aldehyde dehydrogenase activity and CD133+, a marker for chemo-resistance and cancer cell survival.
- Regulation of dormancy and adherence-independent growth within the CSC tumor niche involves the Notch, Wnt and Hedgehog pathways.

Breast Cancer Stem Cells: A New Target for Therapy, Federici, Espina, Liotta & Edmiston, Oncology 2011; 25 (1): 25-28, 30.

Disulfiram

- Anti-alcoholism drug disulfiram (Antabuse) inhibits aldehyde dehydrogenase, NFκB and TGFβ.
- Glioblastoma stem-like cells induced by hypoxia, via HIF and NFκB, are inhibited by a sustained release formulation of disulfiram.
- *The epithelial mesenchymal transition can even be reversed*.
- Reverses chemo-resistance and inhibits invasion and metastasis in a variety of cancers in cell and mouse models.
- Used off-label for glioblstoma multiforme, along with copper gluconate.
- Rx: Patients must abstain from alcohol for at least 12 hours before use. Dosed at up to 500 mg p.o. for 1-2 weeks, maintain at 125-500 mg (usually 250 mg eg 125 mg bid).

Disulfiram Inhibits TGF-Induced Epithelial-Mesenchymal Transition and Stem-Like Features In Breast Cancer via ERK/NF-κB/ Snail Pathway, Han, Wu, Chang, et al., Oncotarget 2015; 6 (38): 40907-40919.

Disulfiram Targets Glioblastoma-Stem-Like cells in vitro and in vivo, Azar, Kannappan, Liu, et al., Neuro-Oncol. 2018; 20 (suppl. 1, 31): p. i20.

- The most common side effect of **disulfiram** includes drowsiness, fatigue and metallic or garlic-like aftertaste.
- Disulfiram is also associated with impotence, blurred vision, skin discoloration, inflammation of the skin, increased heart rate, and mental changes.
- During the first three months of therapy, monitor and test liver function.
- Disulfiram may react with small amounts of alcohol - cold syrups, tonics, and mouthwashes. Patients should not even use topical preparations that contain alcohol such as perfume and after-shave lotion. The signs of alcohol ingestion: flushing, headache, nausea, vomiting, abdominal pain.
- Disulfiram can make cisapride, benzodiazepines, astemizole, cyclosporine, erythromycin, and cholesterol-lowering drugs called statins more toxic. Disulfiram in combination with isoniazid, MAO inhibitors (such as phenelzide or tranylcypromine), metronidazole, omeparazole and tricyclic antidepressants may cause adverse CNS effects. Disulfiram may raise the concentrations of the medications theophylline and phenytoin in the body. Patients on warfarin are at an increased risk of bleeding. Disulfiram should never be used with tranylcypromine and amprenavir oral solution.
- CI: psychosis, MI, CVD, seizures, hypothyroid, nephritis, LV failure.

Metformin Hurts Cancer

- Inhibits **P13-kinase / mTOR/ akt** signaling,
- Inhibits **MAPK** involved in glutaminolysis.
- *Selectively* blocks growth of **OXYPHOS**-dependent cancer stem cells in breast, ovarian and endometrial cancers.

Metformin Against Cancer Stem Cells Through the Modulation of Energy Metabolism: Special Considerations on Ovarian Cancer, Kim, Suh, Kim, et al., Biomed. Res. Int. 2014; 132702.

Metformin in Cancer, Mallik & Chowdhury, Diabetes Res. Clin. Pract. 2018; 143: 409-419.

Targeting Cancer Stem Cells with Dietary Phytochemical - Repositioned Drug Combinations, Chan, Chen & Fong, Cancer Lett. 2018; 433: 53-64.

Metformin Inhibits the Inflammatory Response Associated with Cellular Transformation and Cancer Stem Cell Growth, Hirsch, Iliopoulos & Kevin Struhl, PNAS 2013: 110 (3): 972-977.

Metformin Treatment Exerts Antiinvasive and Antimetastatic Effects in Human Endometrial Carcinoma Cells, Tan, Adya, Chen et al., J. Clin. Endocrinol. Metab. 2011; 96 (3): 808-816.

The Influence of Antidiabetic Medications on the Development and Progression of Prostate Cancer, Hitron, Adams, Talbert & Steinke, Cancer Epidemiol. 2012 Mar 12. [Epub ahead of print]

Metformin Selectively Kills CSCs

- Metformin **selectively inhibits cancer mesenchymal stem cells**.
- Blocks mitochondrial stage 2 complex 1 oxidative phosphorylation.
- Inhibits **NFkB**.
- Inhibits **IGF-1.**
- Inhibits **IL-6**.
- Metformin activates the enzyme AMPK (5'AMP-activated protein kinase) which directly stimulates mitogenesis.

Metformin as an Anti-Cancer Agent: Actions and Mechanisms Targeting Cancer Stem Cells, Saini & Yang, Acta Biochim. Biophys. Sin. (Shanghai), 2018; 50 (2):133-143.

Metformin Inhibits the Development, and Promotes the Resensitization, of Treatment-Resistant Breast Cancer, Davies, Lobanova, Dawicki, et al., PLoS One 2017; 12 (12): e0187191.

Metformin Selectively Target Cancer Stem Cells, and Acts Together with Chemotherapy to Block Tumor Growth and Prolong Remission, Hirsch, Iliopoulos, Tsichlis & Struhl, Cancer Res. 2009; 69 (19): 7507-7511.

Metformin: An Emerging New Therapeutic Option for Targeting Cancer Stem Cell and Metastasis, Rattan, Fehmi & Munkarah, J. Oncolo. 2012; 2012:928127.

Interventions to activate AMPK

i. Exercise
ii. Resveratrol
iii. Curcumin
iv. Quercetin
v. Metformin
vi. Berberine
vii. Green tea EGCG

Metformin Inhibits the Inflammatory Response Associated with Cellular Transformation and Cancer Stem Cell Growth, Hirsch, Iliopoulos & Kevin Struhl, PNAS 2013: 110 (3): 972-977.

Berberine and Berberine-Containing Plants As Antineoplastic Agents, Tang, Feng, Wang, et al., J. Ethnopharmacol. 2009; 128: 5-17.

Exercise in Prevention and Management of Cancer, Newton & Galvao, Curr. Treat. Opt. Oncol. 2008; 92 (3): 135-146.

The Anti-Obesity Effect of Quercetin is Mediated by the AMPK and MAPK Signalling Pathways, Ahn, Lee, Kim, et al., Biochem. Biophys. Res. Comm. 2008; 373 (4): 545-549.

Curcumin for CSCs

- **Curcumin** acts upon **NFκB**, inhibits the **Sonic Hedgehog** signaling pathway, and many other known targets for the regulation of cancerized stem cells.
- Curcumin significantly reduces aldehyde dehydrogenase activity.
- Curcumin targets drug efflux resistance in CSCs via P-gp, ABCG2 (MXR) and MRP1.
- Only curcumin inhibits both tyrosine-dependent kinases and serine-threonine-dependent kinases. This remarkable property prevents cancer cells developing resistance to its effects.
- It is an excellent candidate for *post-surgical inflammation* management.

Targeting Cancer Stem Cells With Phytochemicals, Kawasaki, Hurt, Mistree & Farrar, Molec. Interv**.** 2008; 8 (4): 174-184.

Elimination of Colon Cancer Stem-Like Cells by the Combination of Curcumin and FOLFOX, Yu, Kanwar, Patel, et al., Transl. Oncol. 2009; 2 (4): 321-328.

Curcumin inhibits cancer stem cell phenotypes in ex vivo models of colorectal liver metastases, and is clinically safe and tolerable in combination with FOLFOX chemotherapy, James, Iwuji, Irving, et al., Cancer Lett. 2015; 364 (2):135-141.

Targeting Breast Stem Cells with the Cancer Preventive Compounds Curcumin and Piperine, Kakarala, Brenner, Korkaya, et al., Breast Cancer Res. Treat. 2009 [Epub ahead of print] PMID: 19898931.

Curcumin Prescribing

- Contraindicated in severe iron deficiency, reduces iron absorption.
- Contraindicated in biliary obstruction, jaundice, hepatitis.
- Contraindicated in tobacco addicts.
- Rare mild thrombocytopenia. While labeled by some as an anticoagulant, all it really does is slightly increase prostacyclin PGI-2 levels, reducing risk of unprovoked clots. This is what you would expect with reduced endovascular inflammation.
- These products can stain clothing and countertops.

Cai, Sun, Zheng, et al., *Curcumin Attenuates lncRNA H19-Induced Epithelial-Mesenchymal Transition in Tamoxifen-Resistant Breast Cancer Cells*, Molec. Med. Rep. 2012; 23: 13.

Biological Effects of Curcumin and its Role in Cancer Chemoprevention and Therapy, Singh & Khar, Anticancer Agents Med. Chem. 2006; 6 (3): 259-270.

Effects of Curcumin on Stem-Like Cells in Human Esophageal Squamous Carcinoma Cell Lines, Almanaa, Geusz & Jmasbi, BMC Compl. Alt. Med. 2012; 12:195.

Vitamin C Kills CSCs

- Vitamin C induces stem cell differentiation in brain cancer cells.
- Hepatocellular carcinoma stem cells highly express sodium-dependent vitamin C transporter 2.
- HCC stem cells exposed to high dose vitamin C have an increase ROS resulting in cell cycle arrest and apoptosis *in vitro.*
- HCC patients given HDIVC had increased DFS, HR: 0.622, $p < 0.001$.

Vitamin C Preferentially Kills Cancer Stem Cells in Hepatocellular Carcinoma via SVCT-2, Hongwei, Wang, Fang, et al., npj Precis. Oncol. 2018; 2 (1): doi: 10.1038/ s41698.017.0044.8.

Typical and Atypical Stem Cells in the Brain, Vitamin C Effect and Neuropathy, Nualart, Salazar, Oyarce, et al., Biol. Res. 2012; 45: 243-256.

Immune Approach to Cancer Stem Cells

- Turkey tail mushroom extract PSP inhibits prostate cancer stem cells.

- Lysate of allogenic GBM stemlike cells shows the ability to stimulate dendritic cells, differentiated out of peripheral monocytes, to generate a promising vaccine for glioblastomas.

Chemo-Preventative Effect of PSP Through Targeting of Prostate Cancer Stem Cell-Like Population, Luk, Lee, Liu, et al., PLoS One 2011; 6: e19804.

Dendritic Cell Vaccine Pulsed with Lysate Derived from an Allogenic GBM Stemlike Cell Shows Promise, Hu, et al., 23rd Annual Mtg. Soc. Neuro-Oncol. Nov. 2018.

Mitochondria, Antimicrobials and Stem Cells

- Erythromycins such as azithromycin, and tetracycines such as **doxycycline,** used to treat cancer-related infections, impacted the growth of tumours such as breast, DCIS, ovarian, prostate, lung, pancreatic, melanoma, and glioblastoma.
- *In vitro* five classes of **antibiotics kill cancer stem cells** in 12 cancer cell lines. These disrupt bacteria-like mitochondrial and ribosomal membranes and inhibit mitogenesis:erythromycins, tetracyclines, glycylcyclines, and chloramphenicol eg azithromycin, **doxycycline**, tigecycline; and the helminthic pyrvinium pamoate.

Barbie & Kennedy, *Doxycycline: New Tricks for an Old Drug,* Oncotarget 2015; 6 (23): 19336-19337.

Vitamin C and Doxycycline: A Synthetic Lethal Combination Targeting Metabolic Flexibility in Cancer Stem Cells (CSCs), De Francesco, Bonucelli, Maggliolini, et al., Oncotarget 2017; 8 (40): 67269-67286.

Regression of Ocular Adnexal Lymphoma After Chlamydia psittaci-*Eradicating Antibiotic Therapy,* Ferreri, Ponzoni, Guidoboni, et al., J. Clin. Oncol. 2005; 23 (22): 5067-5073

Antibiotics That Target Mitochondria Effectively Eradicate Cancer Stem Cells, Across Multiple Tumor Types: Treating Cancer Like an Infectious Disease, Lamb, Ozsvari, Lisanti, et al., Oncotarget 2015; 6 (7): 4569-4584.

Most Promising Agents to Control Cancer Stem Cells

- Control post-op inflammation – boswellia, curcumin, berberine, grapeseed.
- Reduce and/or block opiates - cannabis, LDN.
- Control hypoxia – alkaline diet, increase K to Na ratio, exercise, fresh air.
- Curcumin.
- Quercetin.
- Vitamin A.
- Metformin (Berberine?).
- Thymoquinone from blackseed *Nigella sativa.*
- Disulfiram.

The Use Of Natural Products to Target Cancer Stem Cells, Taylor & Jabbarzadeh, Am. J. Cancer Res. 2017;7(7): 1588-1605.

Other possible agents for stem cell modulation:

- Mistletoe lectins – *Viscum album* – SQ, IV, PT.
- Cranberry OPC's.
- Green tea EGCG.
- R-alpha lipoic acid.
- Indole-3-carbinol/ diinolylmethane.
- Vit. C.
- Caloric/protein/methionine restriction.

Satheesh, Samuel & Büsselberg, *Combination Therapy with Vitamin C Could Eradicate Cancer Stem Cells*, Biomolecules 2020: 10, 79.

Cancer Stem Cell Theory and the Warburg Effect, Two Sides of the same Coin? Pacini & Borziani, Int. J. Mol. Sci.2014; 15: 8893-8930.

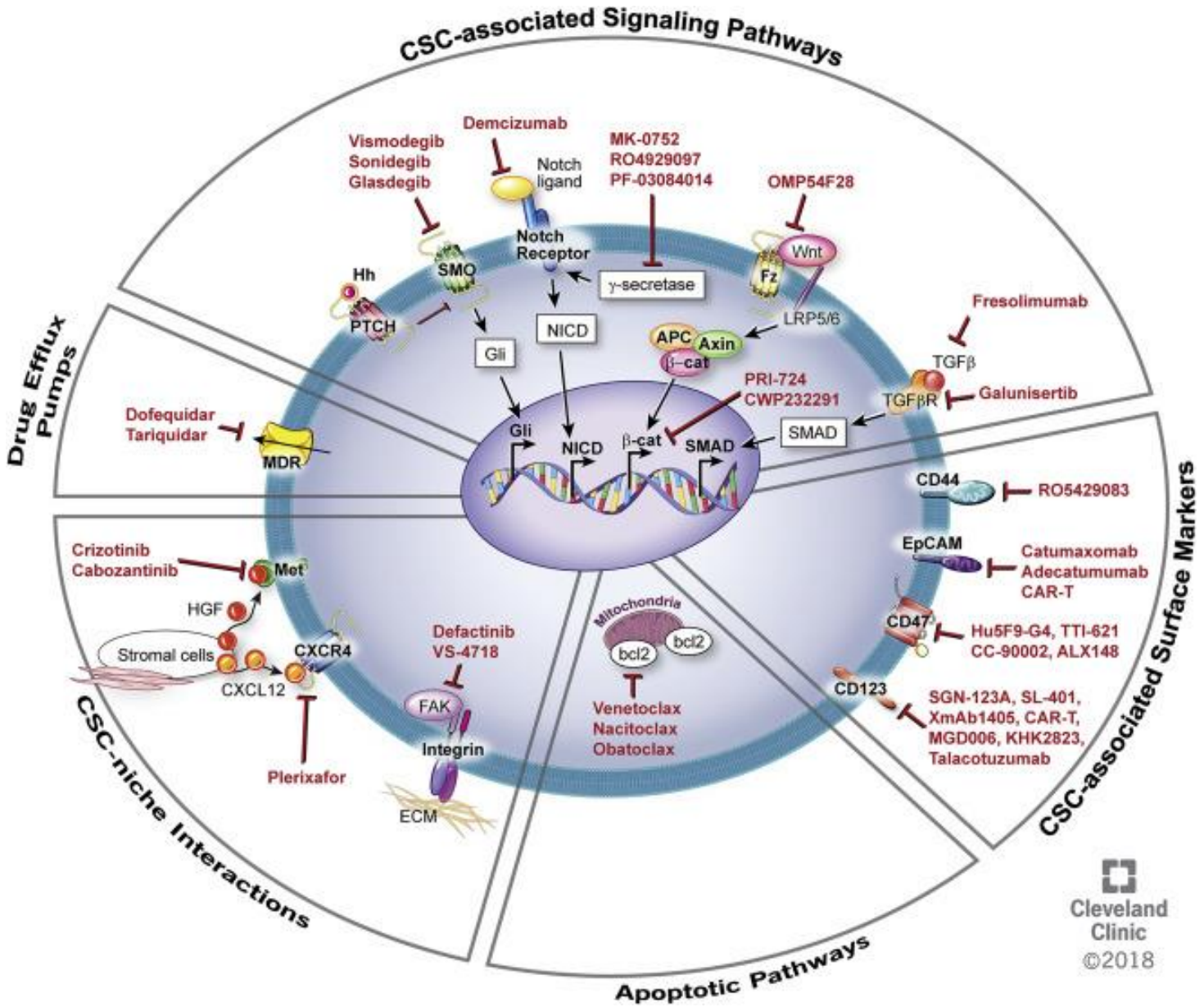
Targeting Cancer Stem Cells With Natural Products, Burnett, Newman & Sun, Curr. Drug Targets 2012; 13 (8): 1054-1064.

Anticancer Phytometabolites Targeting Cancer Stem Cells, Torquato, Goettert, Justo, et al., Curr. Genom. 2017; 18: 156-174.

Targeting cancer stem-like cells using dietary-derived agents – Where are we now?, Khan, Kamokar, Howell, et al., Mol. Nutr. Food Res. 2016 doi: 10.1002/mnfr.201500887.

Natural Products That Target Cancer Stem Cells, Moselhy, Srinavasan, Ankhem & Damordan, Anticancer Res. 2015; 35 (11): 5773-5788.

Drugs for Cancer Stemness



Targeting Cancer Stemness in the Clinic: From Hype to Hope, Saygir, Matei, Majeti, et al., Cancer Stem Cell 2019; 24: 25-40.

Summary

- Cancer Stem Cell Theory is not accepted as the complete explanation of cancer growth, metastasis and treatment resistance.
- A number of biochemical mechanisms are known to be involved – EMT, CXCR-4, CXCL-12 fusion chemokine, Wtn/beta-catenin, TGFβ, Notch and Hedgehog signalling, c-Myc, p13K, mTOR, akt, IGF-1, STAT-3, AMPK, PPARϒ, COX2, HIF1, etc.
- Pre-clinical research suggests several natural medicines as candidates for stem cell management in oncology.
- Many of these have human trials showing general benefits in cancer care.
- Stem cell control may be an important aspect of their efficacy, and thus may provide a new perspective on how to prioritize them in patient care, and to identify potential significant synergies.

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