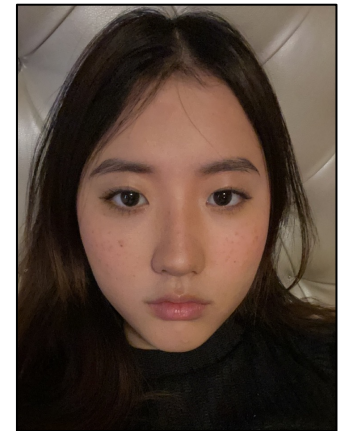


A Novel Approach to Treating **Hormonal Breast Cancer** using **Clinical Database** and **3D ex vivo Model**

(**Metformin** prevents tumor growth and invasion of human **hormonal positive breast cancer** cells via **FOXA1** inhibition)

Christine Song

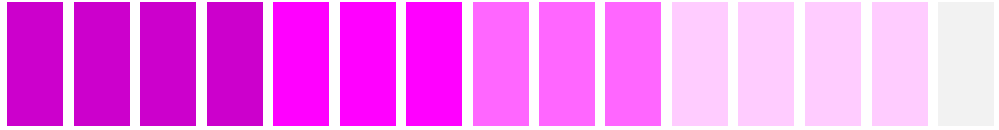
**Mayo High School & Mayo Clinic
(Rochester, MN, United States)**



(Note. Unless otherwise noted, all graphics and images were created by me.)



Breast Cancer



Breast cancer is the second leading cause of cancer death in women. There are four major subsets of breast cancers such as hormonal breast cancer, HER2 positive breast cancer, and triple negative breast cancer (TNBC).

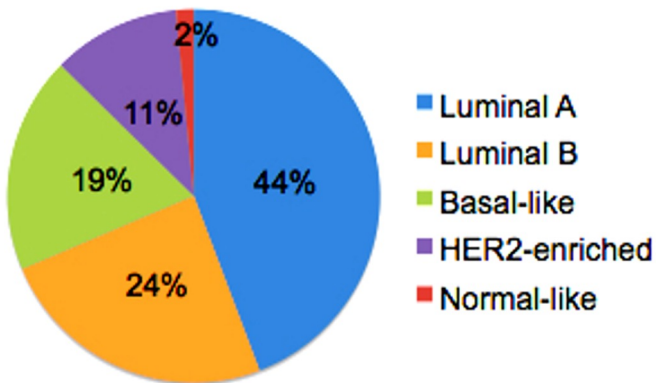
Breast Cancer subtypes & distribution

HR+ HER2-

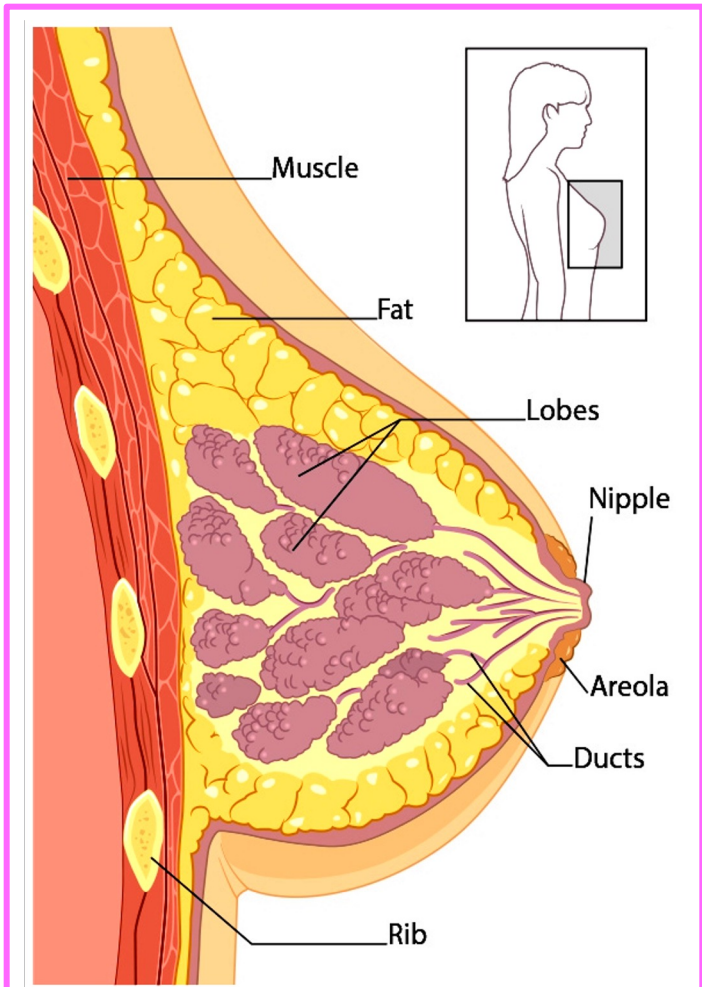
HR+ HER2+

HR- HER2+

TNBC (Triple Negative)



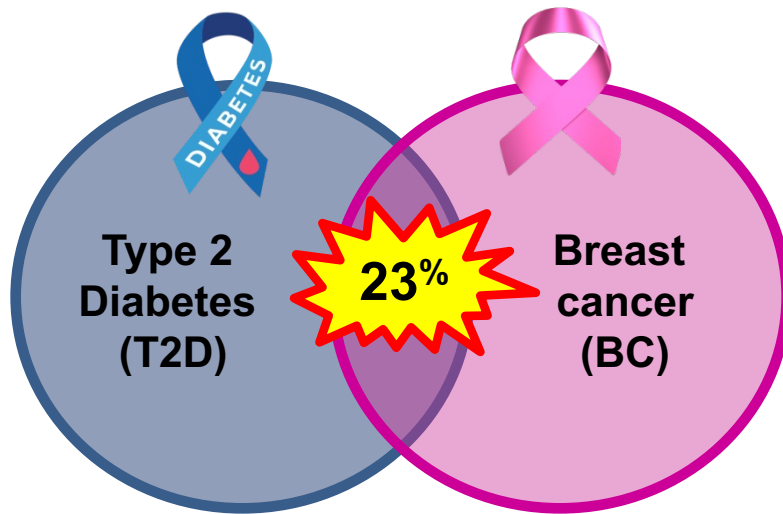
The Cancer Genome Atlas Network (2012)



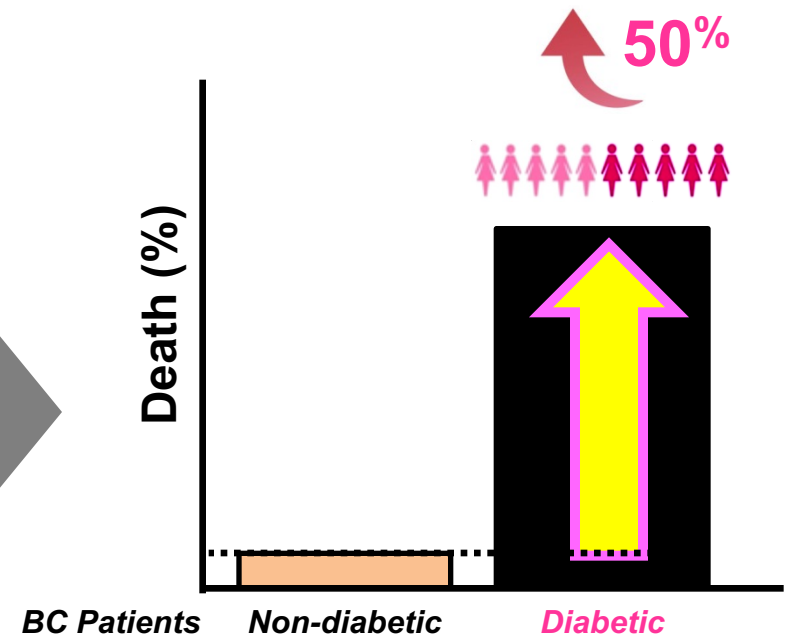
Division of Cancer Prevention and Control (Content source)

Females with type 2 diabetes have a 23% higher risk of developing breast cancer compared to non-diabetic females.

British Journal of Surgery (2013)

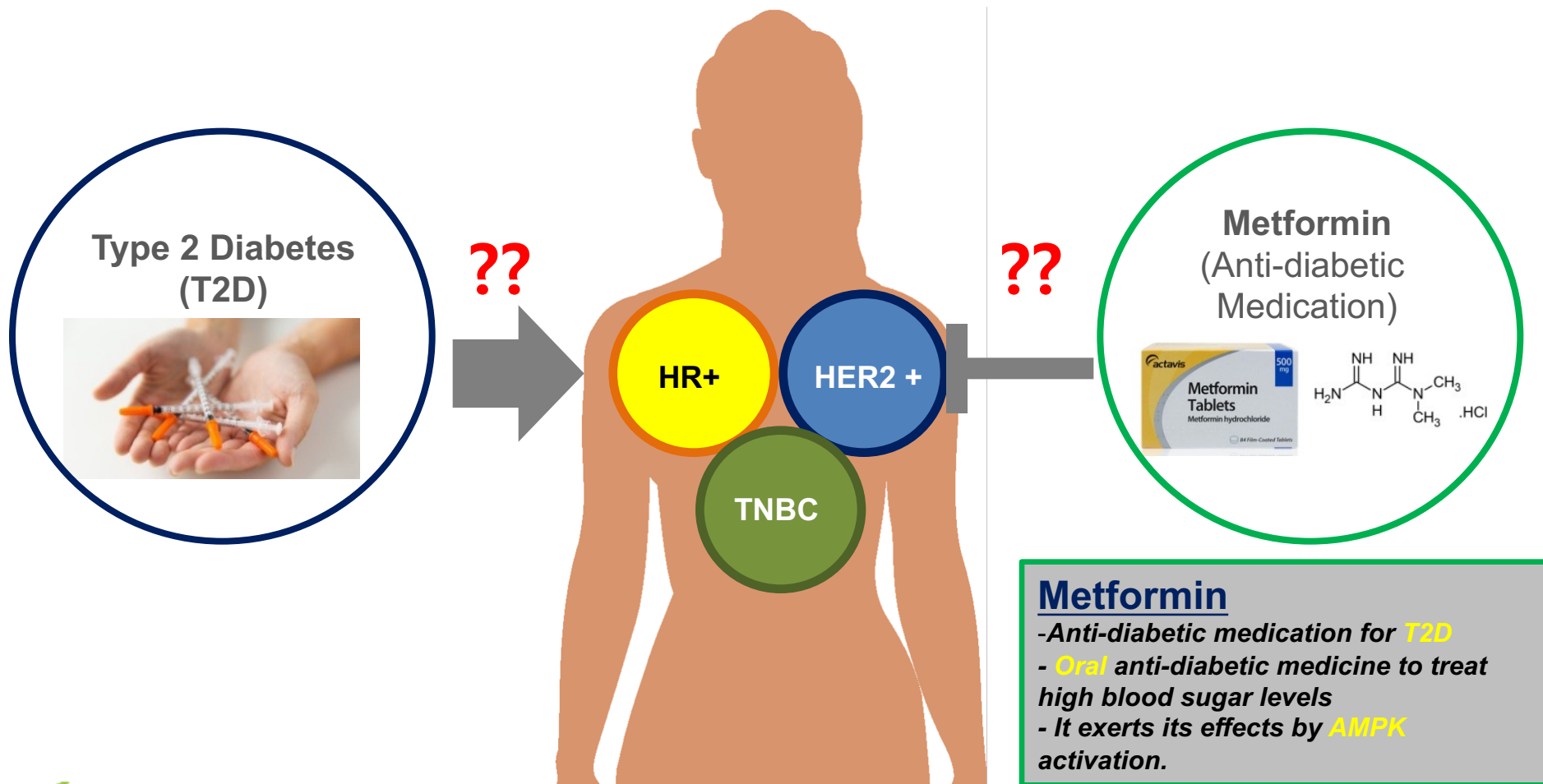


50% higher chance of death for breast cancer patients.



Journal of Clinical Oncology (2011)

- ✓ Diabetes mellitus - a disease that occurs when the pancreatic beta cells do not secrete insulin, or the systemic cells are resistant to insulin
- ✓ Diabetes Classification
Types 1, 2, and gestational diabetes, a small number of people develop certain types of diabetes due to other causes
- ✓ Type 2 diabetes (T2D) - chronic hyperglycemia and cellular insulin resistance
- ✓ T2D causes vascular complications related to retinopathy, nephropathy, neuropathy, and cardiovascular disease



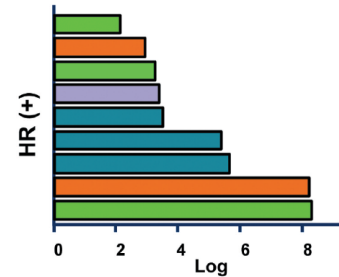
✓ Are all types of breast cancer affected by T2D?

✓ Which breast cancer-associated genes are associated with T2D?

✓ Can the anti-diabetic medication, metformin, treat T2D-related breast cancer?

Previous Study

Which breast cancer subtype is related to Type II Diabetes?



AMPK signaling pathway
 Glutamatergic synapse
 Mitochondria Metabolism
 Peroxisome
 Cell death
 Cellular proliferation
 Apoptosis
 Alcoholism
 Metabolic pathways

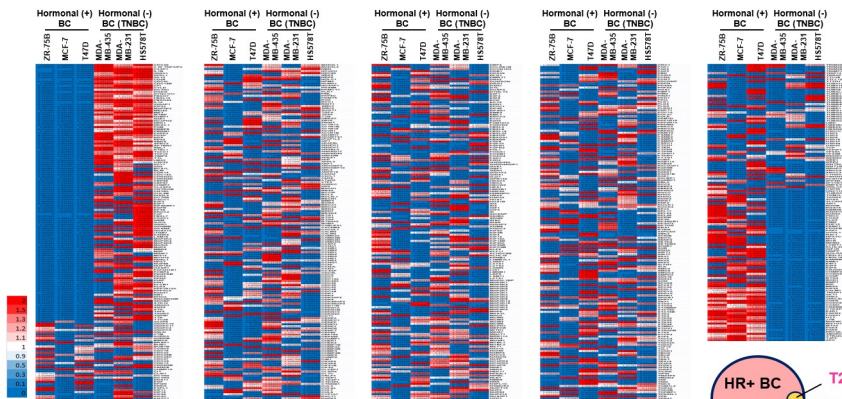
Song C, Kendi AT, Lowe VJ, Lee S. *Anticancer Res.* (2022) Feb;42(2):681-695.

Genes highly expressed in HR+ breast cancer are correlated to cell metabolisms such as the AMPK signaling pathway, mitochondria metabolism, and metabolic pathways. Therefore, the HR+ subtype might have a positive correlation to Type II Diabetes.

Figure 1

Database from 53,805 genes

Is the HR+ breast cancer subtype actually related to Type II Diabetes? If so, how many genes are related?



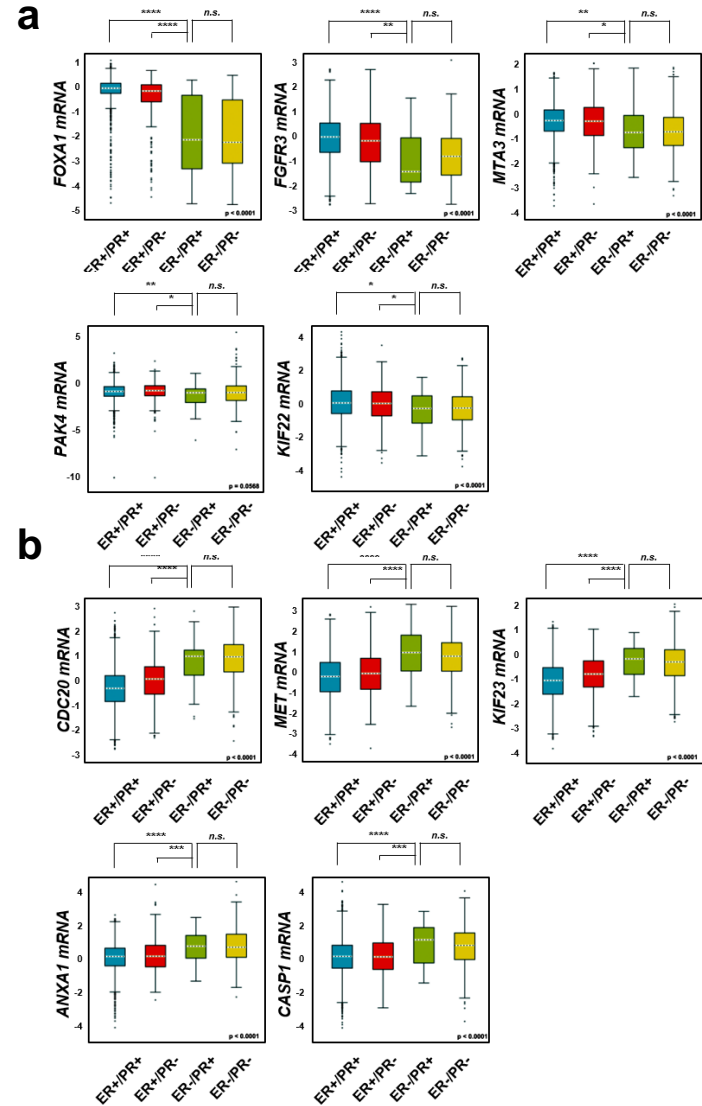
Among 26 genes highly expressed in only the three HR+ breast cancer cell lines, 14 of them were positively related to Type II Diabetes.

Are these genes highly expressed in breast cancer patients?

Figure 2

Database from 4,032 Breast cancer patients

Yes! The diabetes-related genes were highly expressed in only the HR+ breast cancer subtypes (ER+/PR+ and ER+/PR-). However, the genes showed no correlations in other subtypes (ER-/PR+ and ER-/PR-).



Do the diabetes-related genes have a correlation in the HR+ breast cancer subtype?

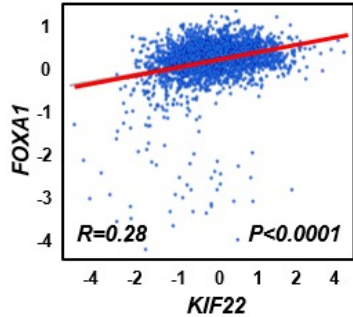
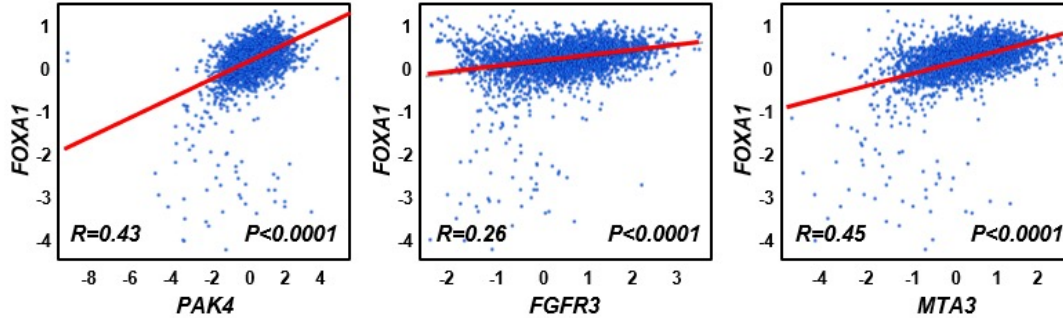
Figure 3

A

Absolutely. The diabetes-related genes, *FOXA1*, showed a significantly positive correlation to other diabetes-related genes, *PAK4*, *FGFR3*, *MTA3*, and *KIF22*, in the HR+ breast cancer subtype.

a

Database from 3,262 Breast cancer patients



b

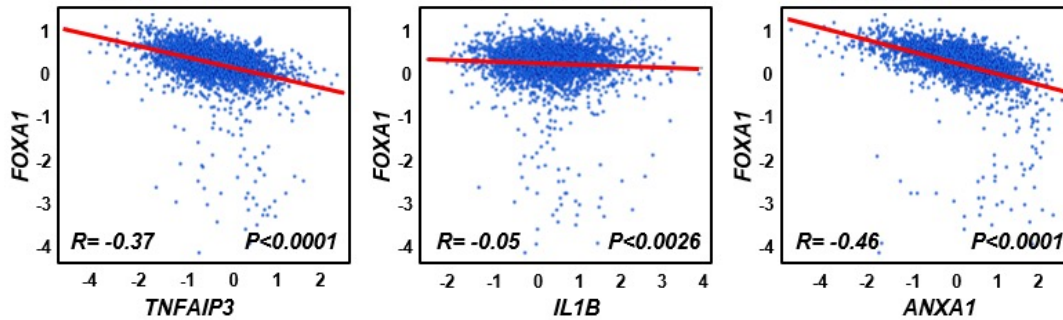


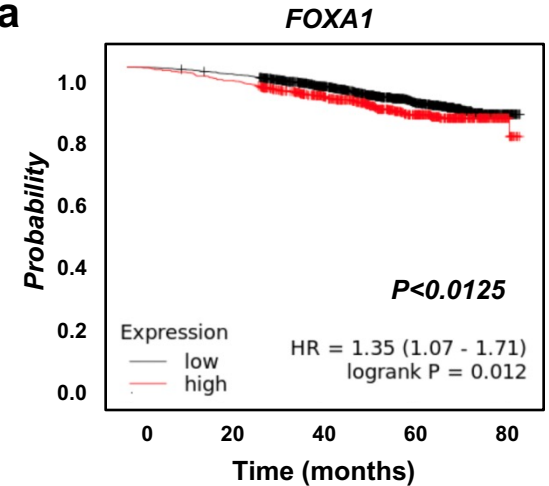
Figure 4

A

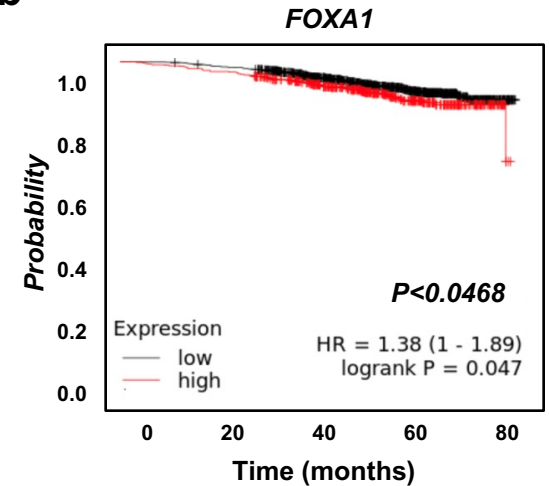
Yes! High expression of the *FOXA1* gene was correlated to worse overall patient survival in HR+ breast cancer patients.

4,981 Breast cancer patients

a



b



Then, does *FOXA1* expression affect breast cancer patient survival?



What function does *FOXA1* have in HR+ breast cancer cell lines?



Figure 5

A

FOXA1 was highly expressed in only the HR+ breast cancer cell lines but not the TNBC cell lines. Furthermore, I found that FOXA1 KO through CRISPR/Cas9 dramatically decreased the tumor cell growth in HR+ breast cancer cell lines, MCF-7 and T47D, using the colony-forming assay.

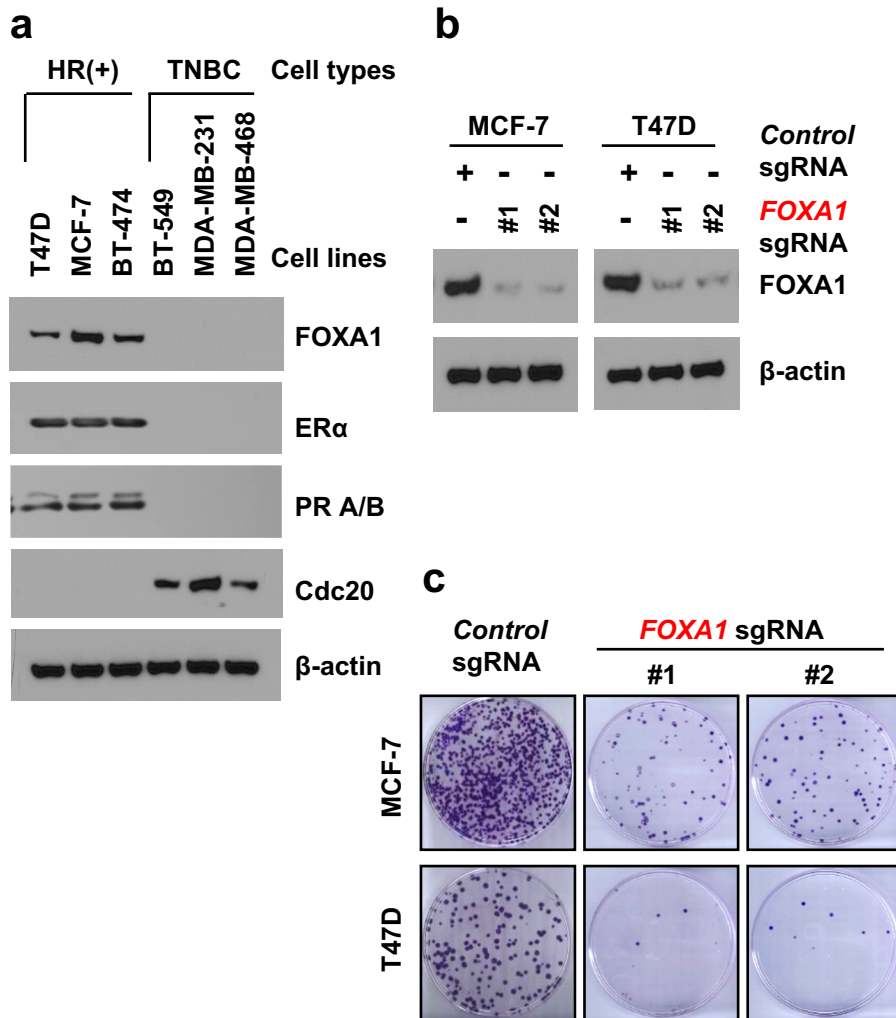
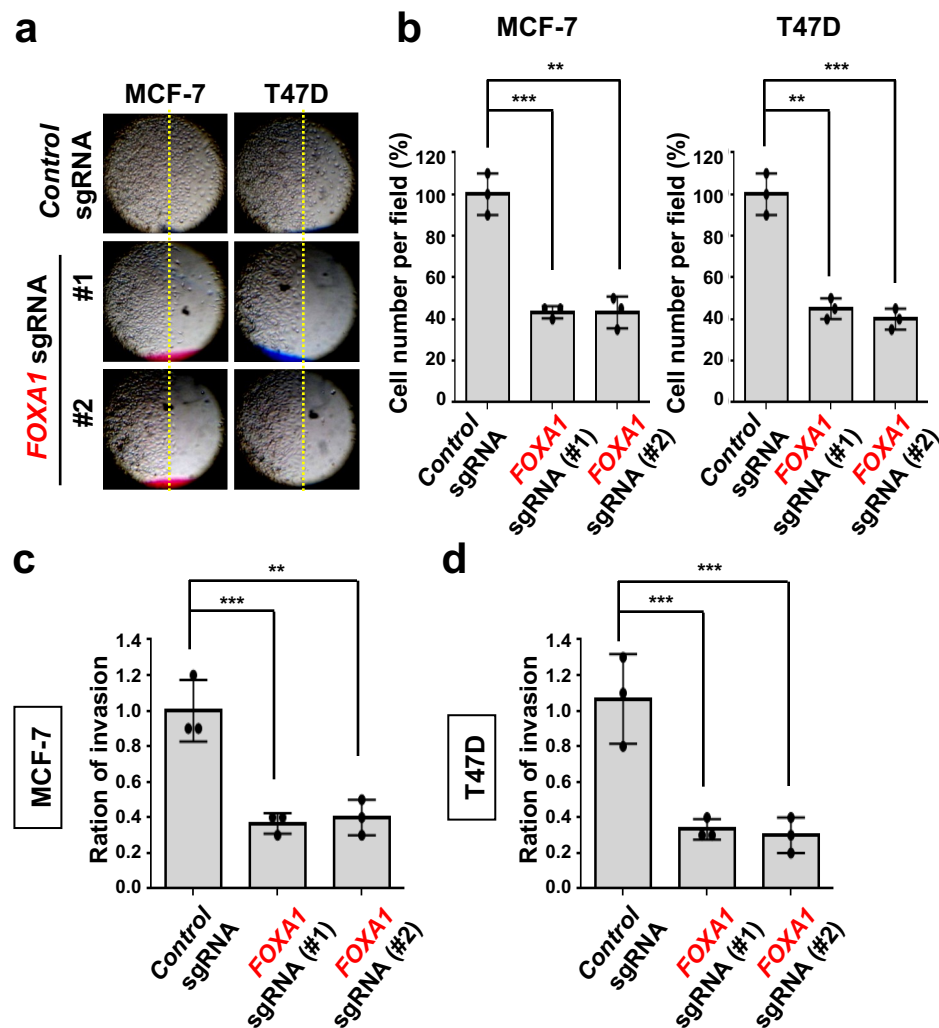


Figure 6

A

Yes! FOXA1 plays a significant role in regulating cell migration and invasion in the MCF-7 cancer cell line. A similar result was attained for the other HR+ breast cancer cell line, T47D.



Does FOXA1 KO affect the metastasis of HR+ breast cancer?



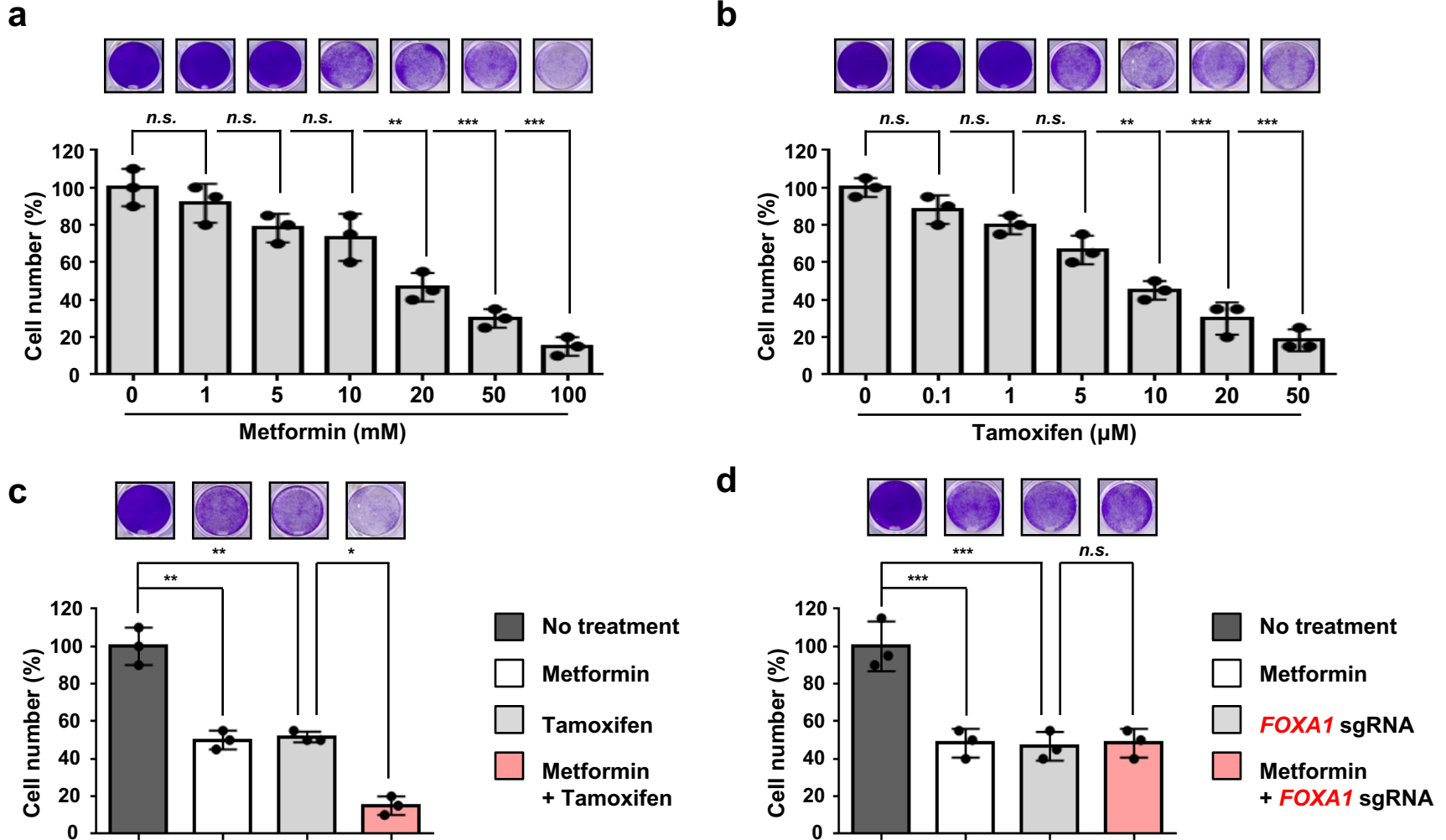
Can diabetes treatment, Metformin, be used to decrease tumor cell growth in HR+ breast cancer?



Figure 7

A

Definitely! Metformin, a diabetic medication, can be used as a medication to decrease tumor cell growth for HR+ breast cancer. Interestingly, the combination of Metformin and Tamoxifen, hormonal therapy for HR+ breast cancer patients, had a synergic effect in blocking tumor cell proliferation.



Can the results of the *in vitro* experiment be replicated *ex vivo* model (3D organoid cell culture system)?

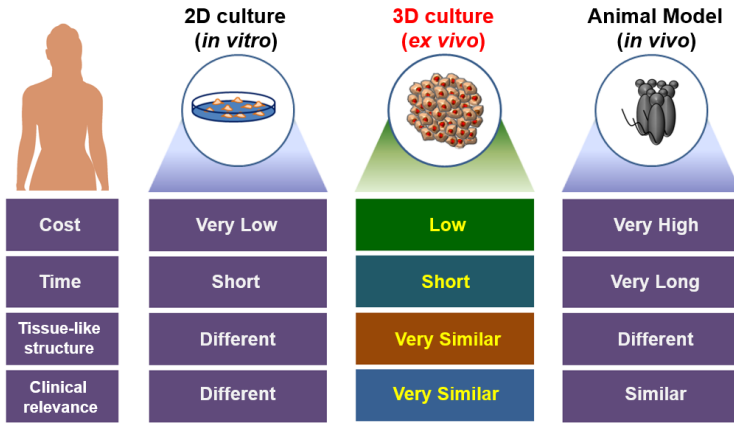


Figure 8

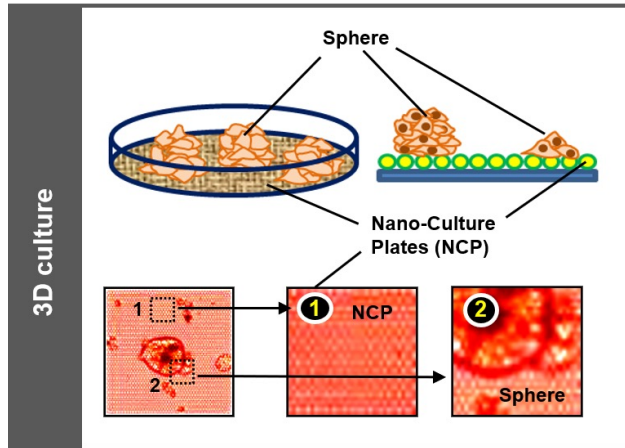
A

Absolutely! I was able to receive similar results *ex vivo*. *FOXA1* KO significantly decreased the number of tumor spheroids in HR+ breast cancer cell lines. Surprisingly, the combination of Metformin and Tamoxifen inhibited tumor cell proliferation more significantly than each individual treatment.

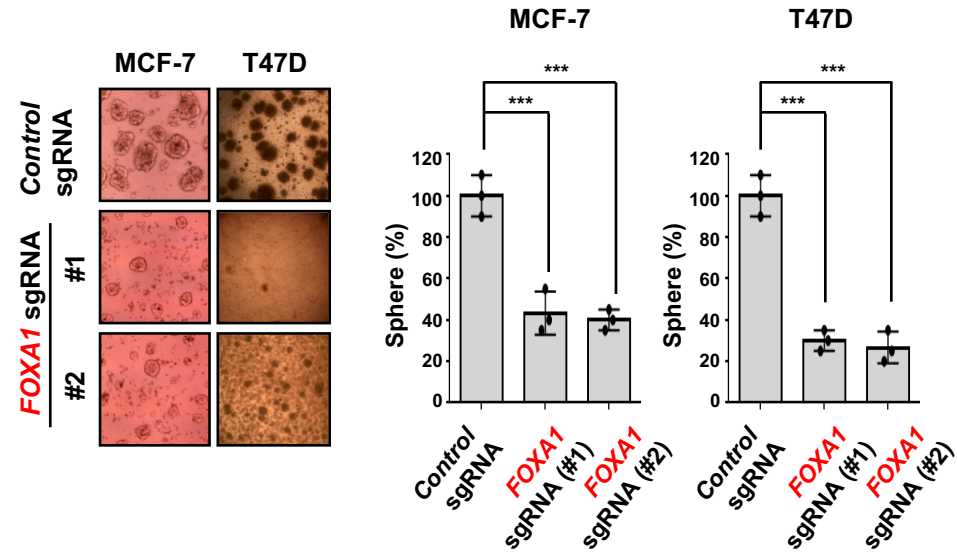
3D Organoid Tissue Culture System (*ex vivo*)



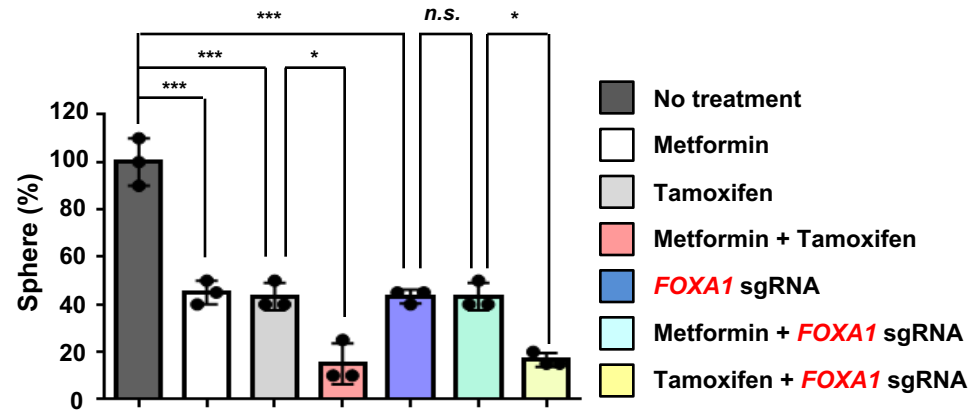
a



b



c



What can I attain from my experiment?

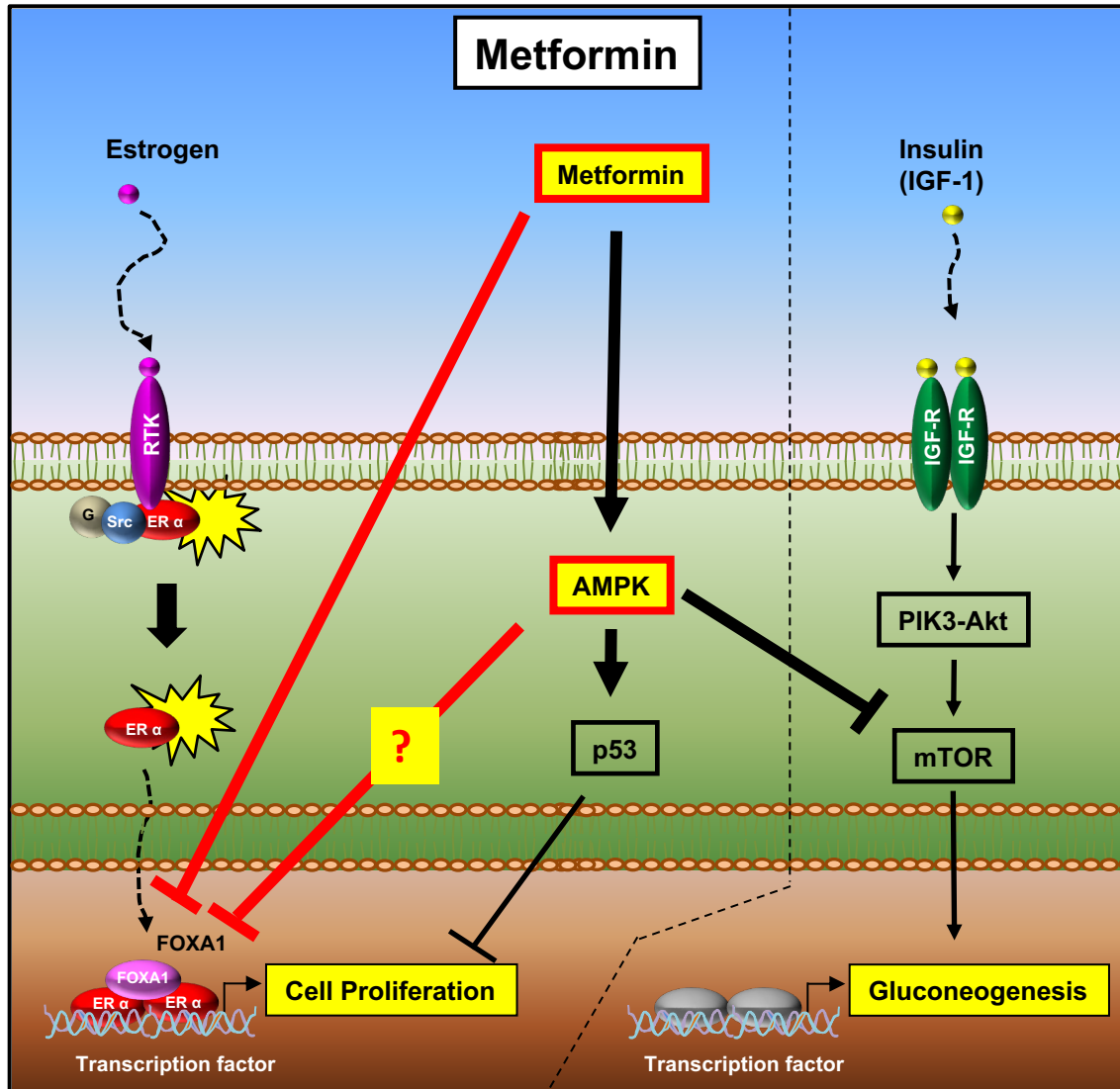


Figure 9

Conclusion & Discussion

A

Metformin, a diabetes treatment, can be used to treat HR+ breast cancer through the regulation of the *FOXA1* gene. The combination of Metformin and Tamoxifen had a synergic effect in decreasing cancer cell proliferation suggesting that the combination might be a novel treatment for HR+ breast cancer patients.



1. Type 2 Diabetes (T2D) had a positive correlation to HR+ BC.
2. Diabetes-related genes, specifically *FOXA1* is highly expressed in only hormonal positive breast cancer and not triple negative breast cancer.
3. High expression of *FOXA1* correlated to a worse overall patient survival for ER+/PR+ breast cancer patients.
4. *FOXA1* KO inhibited HR+ BC cell proliferation and metastasis.
5. Metformin and Tamoxifen combination significantly decreased HR+ BC cell growth.

★ Proposal of new HR+ breast cancer treatment:

1. *FOXA1* could be used as a novel gene target therapy for HR+BC.
2. The combination of Metformin and Tamoxifen might be a novel treatment for HR+ breast cancer patients.

★ Further Studies:

1. Study the correlation between *FOXA1* and the AMPK pathway.
2. Completion of this study through an animal study/preclinical trial (side effect prevention).
3. *FOXA1* relation to other breast cancer subtypes.

Materials & Methods

Cancer data collection and processing

Cells and cell lines and reagents

Gene silencing

Western blot

Colony Forming Assay

Migration and Invasion Assays

3-D Organoid Assay

Statistical Analysis.

Risk and Safety.

We will be using cancer cell lines under the Mayo Clinic policies. Drs. Lowe and Lee will help in the experiment. The BSL level of our experiment is a 0.

References

1. "What Is Diabetes?" Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, 16 Nov. 2021, <https://www.cdc.gov/diabetes/basics/diabetes.html>.
2. Eketunde, Adenike O. "Diabetes as a Risk Factor for Breast Cancer." *Cureus* vol. 12,5 e8010. 7 May. 2020, doi:10.7759/cureus.8010
3. Breast cancer patients with diabetes more likely to die. [Jun;2016];Peairs K. https://www.hopkinsmedicine.org/news/media/releases/breast_cancer_patients_with_diabetes_more_likely_to_die 2011
4. Wolf I, Sadetzki S, Catane R, Karasik A, Kaufman B. Diabetes mellitus and breast cancer. *Lancet Oncol.* 2005 Feb;6(2):103-11. doi: 10.1016/S1470-2045(05)01736-5. PMID: 15683819.
5. Lohmann AE, Goodwin PJ. Diabetes, metformin and breast cancer: a tangled web. *Ann Oncol.* 2021 Mar;32(3):285-286. doi: 10.1016/j.annonc.2020.12.014. Epub 2021 Jan 29. PMID: 33516777.
6. Jalving M, Gietema JA, Lefrandt JD, de Jong S, Reyners AK, Gans RO, de Vries EG. Metformin: taking away the candy for cancer? *Eur J Cancer.* 2010 Sep;46(13):2369-80. doi: 10.1016/j.ejca.2010.06.012. Epub 2010 Jul 23. PMID: 20656475.
7. Rahman, Ishrat et al. "Type 2 Diabetes, Obesity, and Cancer Share Some Common and Critical Pathways." *Frontiers in oncology* vol. 10 600824. 20 Jan. 2021, doi:10.3389/fonc.2020.600824
8. Song C, Kendi AT, Lowe VJ, Lee S. The A20/TNFAIP3-CDC20-CASP1 Axis Promotes Inflammation-mediated Metastatic Disease in Triple-negative Breast Cancer. *Anticancer Res.* 2022 Feb;42(2):681-695. doi: 10.21873/anticancer.15527. PMID: 35093867.
9. Song C, Lowe VJ, Lee S. Inhibition of Cdc20 suppresses the metastasis in triple negative breast cancer (TNBC). *Breast Cancer.* 2021 Sep;28(5):1073-1086. doi: 10.1007/s12282-021-01242-z. Epub 2021 Apr 3. PMID: 33813687.

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3. **Type 2 Diabetes** - <https://www.bostonmagazine.com/health/2019/04/08/diabetes-cure-research/>
4. **Metformin** - <https://www.abcam.com/metformin-hydrochloride-ampk-activator-ab120847.html>
5. **Pink Ribbon** - https://www.kindpng.com/imgv/iihhbwb_cancer-logo-png-transparent-background-pink-ribbon-png/

My Past Research

Year 1 (2018)

Cure of Breast Cancer: Identifying Specific Genes in Triple Negative Breast Cancer (TNBC) using Database

Year 2 (2019)

Cure of Breast Cancer – Year 2: Discovering New Therapies using Natural Products for a New Characterization of Breast Cancer Subtypes

Cdc20 gene & TNBC

Inflammation inhibitors & TNBC

2018

2019

2020

2021

Inflammation & TNBC

Inflammation inhibitors & ER-/PR+ BC

Year 3 (2020)

Cure of Breast Cancer – Year 3: Discovering Inflammation Inhibitors as a Novel Treatment of Triple Negative Breast Cancer using 3D Organoid Culture System

* American Association for Cancer Research (AACR)
- poster presentation (International Conference)

Year 4 (2021)

Cure of Breast Cancer – Year 4: The First Discovery of New Target Therapy for Aggressive Hormonal Breast Cancer using Clinical Database and 3D Model

* American Association for Cancer Research (AACR)
- poster presentation (International Conference)

* Research article – *Breast cancer* (2021), *Anticancer Res.* (2022)

Year 1

Year 2

Year 3

Year 4

Year 5

ER-/PR- (TNBC)

HR+&TNBC

ER-/PR-(TNBC)

ER-/PR+ (HR+)

ER+/PR+ (HR+)

Mitotic inhibitors

Natural products

Inflammation inhibitors

Caspase-1 Inhibitor

Anti-diabetic Medication

BI-2036
VX-680
ZM447439

Blueberry
Soybean
Saponin

Nec-1, NSA
GW80

Ac-YVAD-CHO
(New)

Metformin
(T2D)