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Using Processing Digital Image Methods For Documenting Tumorogenic Breast Disease Cells

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Abstract

We identified and isolated CD44-CD24/low Lineage tumor cells in eight of nine individuals. In mice, tumors might grow from 100 cells with these traits, but not from thousands with other phenotypes. On each serial passage, tumorigenic cells contributed to the tumor's CD44-CD24/low Lineage tumor-causing cell as well as phenotypically variable no tumor-causing cell groupings. They are called tumorigenic or cancer-initiating cells because they constantly create malignancies, unlike additional cancer cell types. This work proposes a unique approach to identify breast asymmetry as well as tumorigenic cancer cells utilizing extremely efficient digital image processing methods that are not previously used in this research field. Chi-square tests and t-tests were used for categorical as well as continuous data. All p-values under 0.05 proved significant.

Key Words: Breast Cancer, Tumor Cells Identification, Cells Growth Survival, Cell Lines, and Structure, and Cells Removal.

Introduction

Triple-negative invasive breast cancer accounts for 15% of cases, and immunotherapies attacking the ER, PR, along with HER 2 receptors are ineffective. Progesterone may cause classic, nonclassic, or mixed reactions by adhering to mPRs or nPRs. We previously showed that the CCM signaling complex may link nPRs and mPRs into the CmPn signalling networks during PRG-induced activity, which is crucial for nPR(+) breast cancer tumorigenesis [1]. Metastases, or cancer's spread, cause many cancer deaths. Cancer therapy requires accurate and timely metastasis as well as therapy reaction predicting. CTCs discharged into the bloodstream caused metastases, although only a small percentage could spread [2]. During tumorigenesis, cancer cells may become too dependent on backup biological processes for existence; these vulnerabilities might serve as therapeutic targets. Novel synthetic lethality associations may lead to new cancer therapies [3]. They are a result of molecular weaknesses. Tumor formation and progression are linked to lipid metabolism changes in the tumor's the microenvironment, involving cancer cells as well as macrophages. A growing number of lipid metabolism-targeted tumor treatments are successful. Lipids and lipid analogues given exogenously protect against certain cancers, including breast cancer [4]. The most dangerous type of breast cancer is triple-negative. The malignancy's peritumoral stroma and significant a substance called buildup have been linked to a poor prognosis. Thus, a substance called synthesis inhibitors may benefit medicine [5]. Pharmacological manipulation of cancer stem cells is a new therapeutic technique for cancer therapy as well as prevention. Breast cancer cells' embryonic origin may be reduced by targeting the human hepatitis P 450 enzymes CYP4Z1. HET0016, a pan-CYP inhibitor, was used to synthesize several novel N-hydroxyphenylformamidines to construct potent and specific CYP4Z1 inhibitors. The newly synthesis derivatives' CYP4Z1 inhibiting properties and structure-activity correlations were examined [6]. Cancer patients' blood CTCs may be exploited as a non-invasive tumor material source for real-time tumor characteristics study. The Parsortix® PC1 Systems is the initial approved by the Food and medical instrument to collect epitope-independent CTCs with different cell size as well as deformation phenotypes. After metastatic breast cancer patients' peripheral blood CTCs are obtained, it will be utilized in user-validated downstream analysis. [7].

Related Work

Researchers attempted to cure TNBC using CDC20 [8]. In silico molecule docking of huge chemical databases (phytochemicals/synthetic medications) against the CDC20 structure of proteins identified five synthetic pharmaceuticals as well as four phytochemicals as potential hits for the site of action. The compounds were selected based on docking, binding energies, interactions, as well as MM/GBSA scores. After analyzing each hit's ADME profile, they found lidocaine, an artificial aminoamide anesthetic with drug-like properties. They

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reported a dose-dependent improvement in growth inhibition of MDA-MB-231 TNBC cancer cells by lidocaine. It was shown that lidocaine reduced cell survival by stimulating apoptosis as well as G2/M arrest in the cell cycle. Lidocaine also downregulated CDC20 gene expression in TNBC cells. That study recommends lidocaine as an anti-neoplastic medication for TNBC cells and CDC20 as a breast cancer treatment target.

29 MDC The authors of [9] integrated single-cell RNA-Sequencing information from seven cancers to create a comprehensive database of the tumor's microenvironment populations. After distinguishing monocyte-derived macrophages from resident-tissue, they identified a group that resembled it. Also, hypoxia-driven macrophages were shown to be important in TME. By deconvolution of these profiles, five subpopulations were found to be distinct prognostic markers for various cancer types. Large datasets confirmed the association among FOLR2-expressing macrophages as well as poor triple-negative cancers of the breast and ovary outcomes. TREM2, which is frequently employed to identify immunosuppressive tumor-associated macrophages, can't be employed alone to predict cancer risk since macrophages express it variably depending on their polarizing state. That comprehensive MDC atlas advances solid tumor treatment choices by providing insightful data as well as novel analyses.

[10] investigated a potential manufactured lethal relationship between a histone methyl transfer enzyme as well as a Chromodomain Helicase DNA-binding proteins related to in a triple negative breast tumor cell line (Hs578T), which lacks biological targets for treatment. Thus, scientists employed CRISPR-Cas9 genome-editing to introduce indels into the Hs578T cell line's genomes at locations corresponding to SETDB1 as well as CHD4's functional domains. Their primary results were: a) Indels in an exon 22 of SETDB1 created Hs578T more vulnerable to the genetically toxic radiation therapy doxorubicin; b) They found evidence of a synthetic fatality connection between the two genes by sequentially including indels in SETDB1 and CHD4 exons 22 and 23 as well as tracking the proportion of remaining wild-type structures in the generated mixed group of cells. Given TNBC's lack of target molecules, their findings may influence new TNBC as well as additional cancer therapy options.

Ceramide, docosahexaenoic acid, sphingomyelin, as well as palmitic acid were tested for anti-tumor properties on malignant breast cells [11]. Every lipid tested reduced cancerous cell competitiveness in vitro by decreasing proliferation, migration, as well as invasiveness. PA increased anti-tumor effects by inducing apoptosis which decreased cancer cell survival while protecting healthy cells. Additionally, co-culture experiments demonstrated that Cer and PA reduced M2 macrophage immunosuppression as well as accelerated breast tumor cell motility as well as epithelial-mesenchymal transformation. At the molecular level, E-cadherin increased. Their results show that exogenous PA as well as Cer, which target tumor cells with M2 macrophages, may considerably lower breast cancer's tumorigenicity. Their findings suggest that lipids may be biocompatible for the treatment of breast cancer.

The [12] article discusses creep and viscoelastic properties and cytoskeletal architecture in tumorigenic and non-tumorigenic cells. Special strain imaging as well as shear testing techniques was used to study creep and viscoelasticity of single non-tumorigenic and tumorigenic cells. A minimum of 20 distinct cells have been studied in three sites. As the malignancy advanced, actin as well as keratin 18 structural densities declined, leading to increased creep rates and lower Young's moduli and viscosities of tumor-causing (MDA-MB-231) cells. The research shows significant creep and viscoelastic differences between tumorigenic as well as non-tumorigenic cells in the breast. Lognormal distributions match creep rate of strain variations, whereas normal distributions fit statistical shifts in viscoelastic properties. The findings' importance for studying cell behaviors, strain as well as viscoelastic replies, as well as the cell cytoskeleton's role in malignancy formation and progression are discussed.

The study in [13] sought innovative, powerful, as well as chemically distinct inhibitors of its production. DDIT, a thymidine analogous, was shown to suppress hyaluronan synthesis in a new small molecular. That drug inhibits tumor growth more than 4-MU. In particular, DDIT inhibits HAS-produced hyaluronan, preventing cancerous breast cell proliferation, migration, invasion, as well as self-renewal. Hyaluronan biosynthesis inhibitors are emerging, and DDIT may cure breast cancer.

Zanthoxylum zanthoxyloides preparations may include cancer-preventive compounds [14]. Bioassay-guided Z isolation. N-methylatanine, N-methylplatydesminecation, sesamin, as well as skimmianine have been extracted from zanthoxyloides methanol extract. The most active chemical, skimmianine, increased activity of Lucifer by 2.8 percent. Skimmianine as well as other quinolone alkaloids may be used to develop new cancer drugs.

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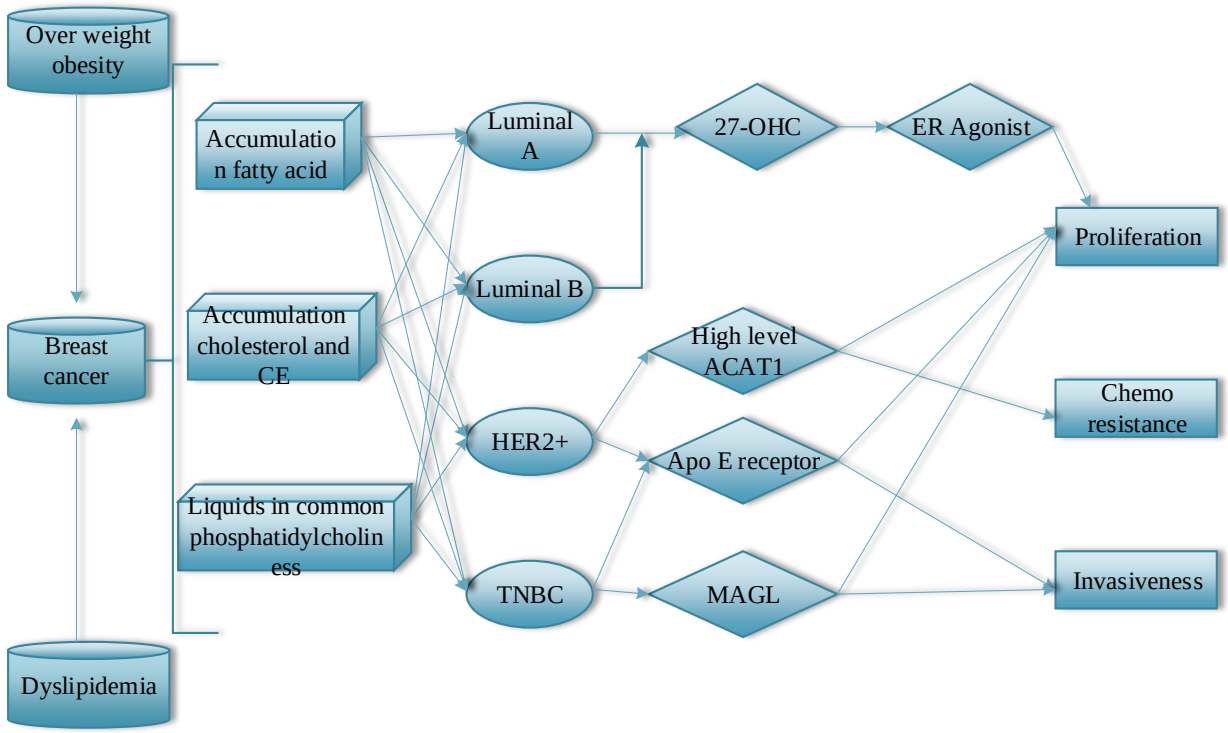
Calocybe indica was studied for its anti-inflammatory and anti-cancer effects [15]. Calocybe indica extract from ethanol was produced. After 72 hours of treatment to 100µg/ml of Calocybe indica gathers, human breast cancer cell (MCF 7) showed a 69.11% reduction in their growth. That study found anti-inflammatory as well as anticancer effects in Calocybe indica ethanol extract.

Breast Cancer Stem Cells



Cancer stem cells (CSCs) may develop into any cell in a cancer sample and share other properties with normal stem cells. They can be detected in tumors or blood malignancies. Thus, CSCs may become malignancies. This may vary from non-tumor-causing cancer cells. The human breast cancer cell lines SK-BR-3, MCF-7, T47D, BT474, MDA-MB-468, and MDA-MB-231 were given by ATCC in Manassas, VA, USA. A medium with HyClone FBS, Gibco 1% penicillin-streptomycin, CellCook CM1007 insulin, plus CellCook CM10085/L necessary amino acids were utilized to develop MCF-7 cells (Gibco cat: 11,095). Cells from different malignancies were cultured in RPMI 1640 (Gibco, cat:11,875) with 10% FBS (HyClone, Utah, USA) and 1% penicillin-streptomycin (Gibco).

Methods And Materials

4.1Breast Cancer Images

Image analysis at tiny magnification is difficult because histological pictures comprise multiple tissues. Learning discrete characteristics from a picture at several magnification levels may help establish a precise diagnosis, but it's difficult. Mammograms, MRIs, DBTs, and ultrasounds are utilized to screen breast.

- *X-rays of breast tissue are used in mammograms (MGs), which are non-invasive. It shows masses with calcifications. It is the most efficient as well as sensitive screening technique because it may identify breast cancer before any symptoms occur, minimizing mortality.*
- *MRI uses powerful magnets as well as radio waves to create detailed images of the breast. Women at elevated risk for cancer of the breast benefit from this technique.*
- *Ultrasound images the breast's interior anatomy using sound waves. It is utilized for high-risk breast cancer patients who can't have an MRI or pregnant women who shouldn't be exposed to MG's x-ray. Also, ultrasonography is often utilized to evaluate women with thick breast tissue.*

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- *Digital Breast Tomosynthesis (DBT) was approved by the Food and in 2011. DBT uses low-dose x-rays to create a more sophisticated mammography. It is 3D mammographic scans that may show masses as well as calcifications with greater clarity, which can help radiologists diagnose dense breasts*.

The two techniques used for identifying breast cancer stem cell lines with tumorigenic potential were (a) cultivating cells in non-adherent non-differentiating circumstances to generate atmospheres as well as (b) sorting cells by surface traits, which includes CD24 and CD44 expression.

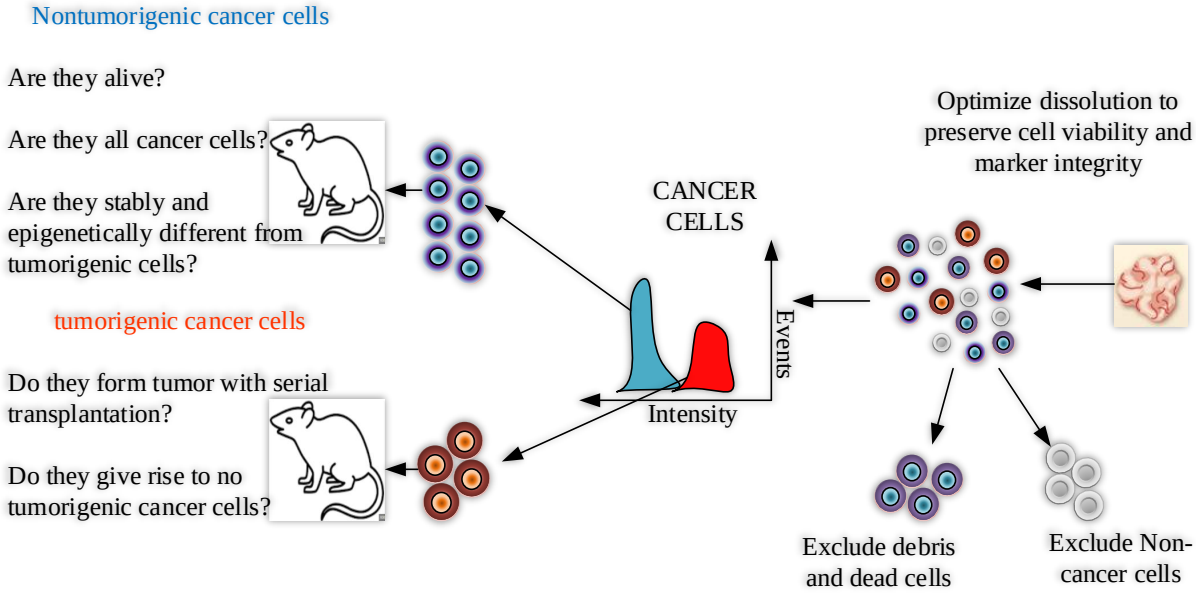

Fig.1. Tumorigenic potential identification

We found many morphometric subgroups of CTCs from breast cancer patients, with one having the greatest tumor-causing potential in vivo having having the lowest rate of cell growth in vitro.

Image Preprocessing

A digital image is a function $f(x, y)$ discrete in brightness as well as space. This function generates brightness by reflecting at every location (x, y).

Image noise removal is the major purpose. Steps can accomplish this:

- Utilizing median filters.
- Set a threshold value to boost visual contrast. A histogram can decide the threshold. HMCLAHE could this.
- Enhance micro calcification difference utilizing dilatation, a morphological operator.
- After pre-processing, picture noise will be removed as well as micro calcification brightness will be boosted, making them visible.

Identification of Breast Asymmetry

It is a key breast cancer diagnostic characteristic. Asymmetry in the picture may be assessed by recording questionable points across one breast to another while decreasing them to comparable points. Asymmetry in the mammographic mammary picture might be detected at the conclusion of this step.

After pre-processing, picture noise would removed as well as tumor genenic contrast will be increased, making them apparent.

The suggested approach will detect breast asymmetry, an early sign of malignancy. The suggested approach would discover tumorgeneic cancer cells early on utilizing processing images as well as categorize them as benign or malignant with high accuracy, yielding true positive as well as genuine negative findings.

Histogram and Thresholding

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A monochrome image's distribution shows the amount of pixels per gray level. Usually, a graph with bars shows the picture pixel counts for each gray level.

The approach splits picture areas into two categories (background as well as object). Binarization is used since thresholds generate a binary picture. Following the thresholding rule, a binary picture T(x,y) is produced from an input picture f(x,y) with N shade of grey:
If $f(x,y) \geq L$, then $T(x,y) = 1$; otherwise, $T(x,y) = 0$, where L is the threshold. This threshold L may be calculated manually or automatically.

Feature Extraction & Classification

Each patient photograph is obtained from a different viewpoint, therefore its characteristics may vary. A patient's previous or future mammogram might also provide tumor information. Thus, at every projection position, a matrix, A(i), comprising 250 pictures as well as 15 textural and statistical attributes, i is the amount of projections.

Texture is the spatial organization and fluctuation of intensity (gray values) in a picture. Texture properties help detect micro-calcification. Two zones may be created from picture capture. The constant thickness zone is the center breast area with virtually consistent thickness. The other type is tissues around the breast's border, where breast geometry reduces thickness. For appropriate area categorization, segmented picture characteristics must be retrieved.

Table.1.

$A(i) =$ images $(205 \times 15$ size $)$	A001 … … A015
	B001 … … . B015
	N001 ..... N015

Results & Discussion

5.1Tumorigenic Breast Cancer Cells Samples

After giving informed consent, eight 2019 and 2020 curable breast cancer patients at Nanfang Hospitals of Southern Medical College had their tumors as well as normal tissue excised. No residual carcinoma cells were seen in the surgical margins, and age, sex, clinical stage, chemotherapy with neoadjuvant therapy, ER, PR, HER2, Ki67, histological category, as well as grade were available for all patients.

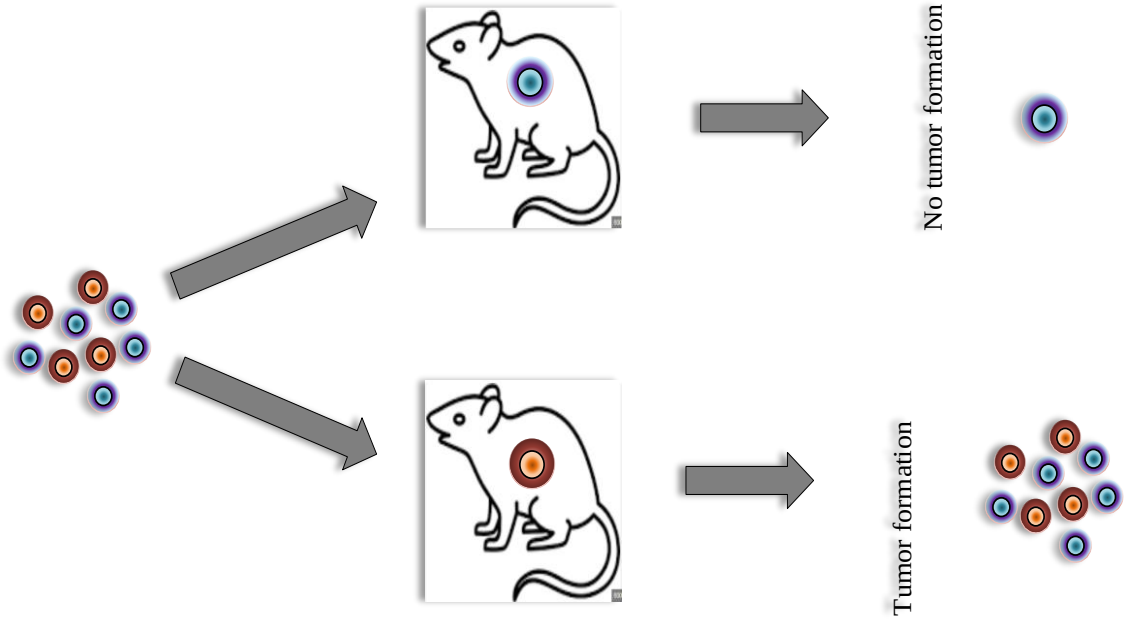

Fig.2. Tumorigenic Breast Cancer formation

Human carcinoma of the breast cell lines with CD44+/CD24- cells have a basal phenotype, not tumor-causing potential. CD44 as well as CD24 expression predicts luminal, basal, or mixed cell shape. Representative CD44 and CD24 flow cytometry dot plots as well as phase-contrast brightfield pictures of each cell line subtype are shown. MCF7 and SUM225 are luminal-like, CD24+ cell; SUM159, SUM1315, as well as MDA.MB.231 are basal-like, CD24- cells; while HMEC and SUM149 are mixed luminal as well as basal cells.

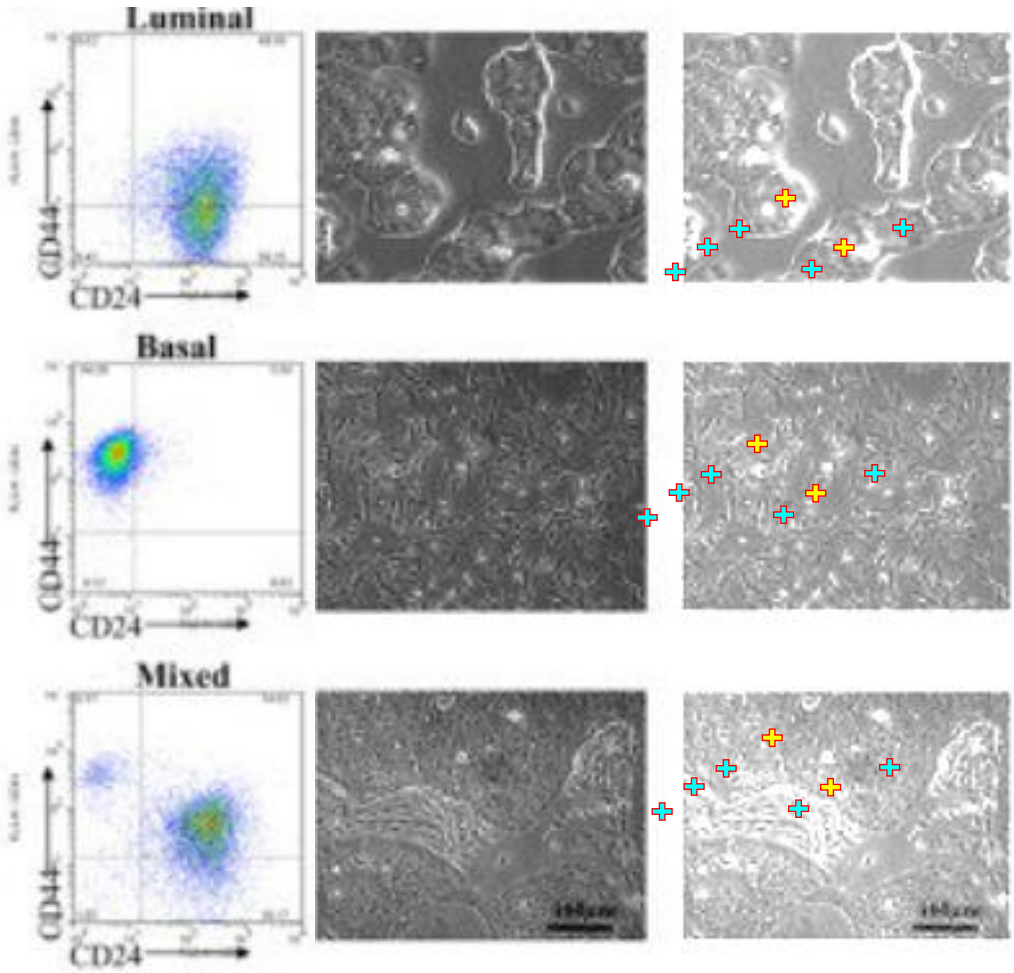

Fig.3. Chronological imaging of to CSC behavior research

Chronological imaging of live cells is essential to CSC behavior research, and we expect C5S-A will prove valuable in time-lapse imaging applications.

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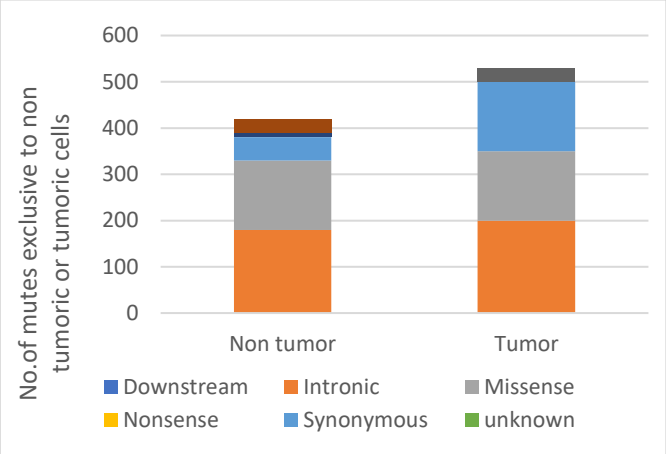

Fig.4.Analysis of tumor and non-tumor

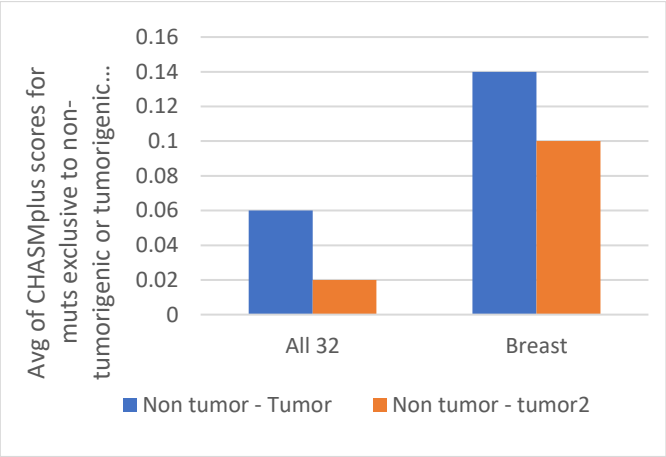

Fig.5.plus score result of tumorigenic

We found that breast cancers had two cell populations: one tumorigenic and one non-tumorigenic. Some tumor-causing carcinoma cells may not multiply due to mitosis-induced chromosomal abnormalities, contributing to their genetic instability. However, given the tumorigenic population was detected in eight of ninth tumor samples, it's plausible to presume that these cancers evolve similarly. The IGS was shown to strongly correlate with both overall and metastasis-free rate of survival in cancer of the breast patients, regardless of preceding clinical or pathological variables ($P<0.001$). The IGS and NIH prognosis criteria were used to classify high-risk breast cancer patients into good or bad predictive groups. Good prognostic patients had an 81% ten-year survival rate, whereas poor predictive patients had 57%.

Conclusion

Normal stem cells may resist chemotherapy due to enhanced BCL-2 family protein levels, membrane transporters such breast cancer treatment-resistant amino acid chains, and multiple resistance to drugs (29–32). Tumorigenic carcinoma cells may generate these proteins to withstand therapy. The cancerous group of cancer cells must be identified to produce more effective cancer therapies. These cells' substances can only be targeted then.

References

1. Ramos-Inza, S., Encío, I.J., Raza, A., Sharma, A.K., Sanmartín, C., & Plano, D. (2022). Design, synthesis and anticancer evaluation of novel Se-NSAID hybrid molecules: Identification of a Se-indomethacin analog as a potential therapeutic for breast cancer. European journal of medicinal chemistry, 244, 114839 .
2. Budianto, M.B., Lubis, H.S., Fadli, M.L., & Nurwidyaningtyas, W. (2023). Check update pattern of tumorigenic vasculature signature based on MMP9 and CXCR4 expression in locally advanced breast cancer. Journal of Pharmacy & Pharmacognosy Research.

3. Brindisi, M., Frattaruolo, L., Sicari, V., Loizzo, M.R., Bedini, G., Rago, V., Tundis, R., & Cappello, A.R. (2022). Santolina pinnata Viv. Exerts Promising Antitumor Activity against Breast Cancer Cells and Anti-Inflammatory Effects in LPS-Stimulated RAW 264.7 Cells. International Journal of Molecular Sciences, 23.
4. Wang, M., Feng, R., Chen, Z., Shi, W., Li, C., Liu, H., Wu, K., Li, D., & Li, X. (2022). Identification of Cancer-Associated Fibroblast Subtype of Triple-Negative Breast Cancer. Journal of Oncology, 2022.
5. Sioud, F., Dhouafi, Z., Lahmar, A., Elgueder, D., & Chekir-ghedira, L. (2022). A Novel Anticancer Effect of Ephedra alata Decne in Breast Cancer Cells. Nutrition and Cancer, 74, 3403 - 3412.
6. Yang, C., Jin, Y., Mei, J., Hu, D., Jiao, X., Che, H., Tang, C., Zhang, Y., & Wu, G. (2022). Identification of icaritin derivative IC2 as an SCD-1 inhibitor with anti-breast cancer properties through induction of cell apoptosis. Cancer Cell International, 22.
7. Ciccioli, M., Moore, R.G., Kim, K.K., Khazan, N., Miller, M.C., & Pailhes-Jimenez, A. (2023). Abstract P1-05-35: Identification of Circulating Tumor Cells captured by the FDA Cleared Parsortix® PC1 System from the Peripheral Blood of Metastatic Breast Cancer Patients using Immunofluorescence and Cytopathological Evaluations. Cancer Research.
8. Das, A., Sharma, H.K., Lather, V., Pandita, D., & Agarwal, P. (2023). Structure-based virtual screening for identification of potential CDC20 inhibitors and their therapeutic evaluation in breast cancer. 3 Biotech, 13.
9. Guimarães, G.R., Maklouf, G.R., Teixeira, C.E., de Oliveira Santos, L., Tessarollo, N.G., Pretti, M.A., Toledo, N.E., da Cruz, J.G., Falchetti, M., Dimas, M.M., Serain, A.F., de Macedo, F.C., Rodrigues, F.R., Bastos, N.C., da Silva, J.L., da Rocha, E.L., Chaves, C.B., de Melo, A.C., Moraes-Vieira, P.M., Mori, M.A., & Boroni, M. (2023). Identification of novel myeloid-derived cell states with implication in cancer outcome. bioRxiv.
10. Moraes-Almeida, M., Sogayar, M., & Demasi, M.A. (2023). Evidence of a synthetic lethality interaction between SETDB1 histone methyltransferase and CHD4 chromatin remodeling protein in a triple negative breast cancer cell line. Brazilian Journal of Medical and Biological Research, 56.
11. He, Y., Rezaei, S., Júnior, R.F., Cruz, L.J., & Eich, C. (2022). Multifunctional Role of Lipids in Modulating the Tumorigenic Properties of 4T1 Breast Cancer Cells. International Journal of Molecular Sciences, 23.
12. Onwudiwe, K.C., Obayemi, J.D., Hu, J., Oparah, J., Onyekanne, C., Nwazojie, C.C., Aina, T., Uzonwanne, V.O., Salifu, A.A., & Soboyejo, W.O. (2021). Investigation of creep properties and the cytoskeletal structures of non-tumorigenic breast cells and triple-negative breast cancer cells. Journal of biomedical materials research. Part A.
13. Karalis, T., Shiau, A.K., Gahman, T.C., Skandalis, S.S., Heldin, C., & Heldin, P. (2022). Identification of a Small Molecule Inhibitor of Hyaluronan Synthesis, DDIT, Targeting Breast Cancer Cells. Cancers, 14.
14. Guetchueng, S.T., Nahar, L., Ritchie, K.J., & Sarker, S.D. (2021). Evaluation of the chemopreventive effect of selected medicinal plants extracts via induction of the Nrf2 in a modified model of breast cancer cells: identification of bioactive lead compounds. European Journal of Cancer Prevention, 31, 50 - 53.
15. S., M., N., R., V., S., K., P., Dass Prakash, M.V., & N., M. (2021). GC-MS Identification of Anti-inflammatory and Anticancer Metabolites in Edible Milky White Mushroom (Calocybe indica) against Human Breast Cancer (MCF-7) Cells. Research Journal of Pharmacy and Technology.