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DIFFERENT CELL LINES SERVE DIVERSE ROLES IN IN-VITRO STUDIES OF DRUGS

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ABSTRACT

Cell lines have been pivotal tools in various fields of research, including cancer biology, drug discovery, developmental biology, and regenerative medicine. This review provides a comprehensive overview of different types of cell lines, their applications, and associated challenges. The review begins by categorizing cell lines based on their origin, including immortalized cell lines, primary cell lines, and stem cell lines. Immortalized cell lines, derived from tumor or transformed cells, offer advantages in long-term culture but may exhibit genetic alterations not present in primary cells. Primary cell lines, obtained directly from tissues, closely resemble in vivo conditions but have limited replicative capacity. Stem cell lines, such as embryonic stem cells and induced pluripotent stem cells, possess self-renewal and differentiation potential, making them valuable for regenerative medicine and disease modelling.

The applications of cell lines span a wide range of research areas. Cancer cell lines, in particular, are extensively used for studying tumor biology, drug screening, and personalized medicine. Additionally, cell lines derived from specific tissues or organs contribute to understanding organ development, physiology, and pathology. Stem cell lines hold promise for cell-based therapies, tissue engineering, and studying developmental processes. Despite their widespread utility, cell lines pose several challenges. Contamination, misidentification, and genetic drift are common issues that can compromise research integrity. Furthermore, discrepancies between cell lines and in vivo models may limit translational relevance. Standardization of cell line authentication, culture conditions, and experimental protocols is essential to address these challenges and ensure reproducibility across studies.

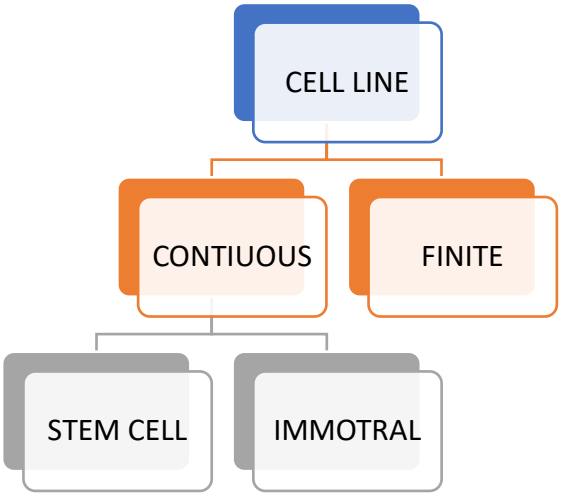
Keywords: Cell lines, immortalized cell lines, primary cell lines, stem cell lines, cancer biology, drug discovery, regenerative medicine, challenges, applications.

INTRODUCTION

Cell lines are widely used in both academic and commercial contexts for experimental biomedical research. Although the first immortalized cell line was created in 1943 using mouse L cells, most people consider the development of the HeLa cell line in 1951, derived from Henrietta Lacks' cervical carcinoma, to mark the beginning of the age of cell lines. Since then, there have been exponential growths in both the quantity and variety of cell lines available in the world. The creation of hybridomas, or hybrid cell lines that produce mAb, by Milstein and Köhler in 1975, the derivation of ESC lines from mice in 1981 and from humans in 1998, and the development of induced pluripotent stem cells (iPSCs) by Takahashi and Yamanaka in 2006 were significant turning points [1].

Cell lines' role in the reproducibility challenge can be attributed to two distinct problems. The first is the misidentification/contamination issue with cell lines. Our data indicates that there are approximately 50 organizations that are significant players in the cell line distribution market (i.e., each distributing more than 10 different cell lines). They are not considered as bioinformatics resources, but they do serve as a de facto information source for the cell lines they provide [2]. However, they rarely employ sound information management techniques, and they don't provide any tools that would enable their data to be integrated with other resources. Additionally, the data they present on their product pages is frequently inaccurate, highly variable, written in free style, and never standardized. We are working with several cell-line collections, and as will be covered in the section on annotation strategies, they are gradually refining their methods. These resources also have the disadvantage of only offering information on the cell lines they disseminate by design, which fragments the cell line information space into as many silos as there are cell line collections [3].

TYPES OF CELL LINES



1.BREAST CANCER CELL LINES

INTRODUCTION

Breast cancer is a very heterogeneous illness that includes a variety of genetically and epigenetically unique conditions with different clinical characteristics [4]. Since breast cancer cell lines might provide an endless supply of homogenous self-replicating materials utilizing straightforward yet conventional media and techniques, a significant amount of current knowledge on breast carcinomas is acquired from in vivo and in vitro investigations using these cells [5]. Before getting any results that are therapeutically useful, it is crucial to determine if these cell lines accurately reflect the heterogeneity of comparable malignancies and capture their molecular characteristics. Despite the conclusion that breast cancer cell lines are largely representative of breast carcinoma, with ER and HER2 serving as crucial stratifies for their classification, ongoing research has revealed dramatic changes in genetic and epigenetic makeup during the initial establishment of the cell line and subsequent serial passaging, raising the possibility that the resulting cell lines underwent significant evolution from the original tumours [6]. However, there are extremely few cell lines that are frequently utilized for research on breast cancer; about two-thirds of the cell lines used in related studies are MCF7, T47D, and MDAMB231 [5].

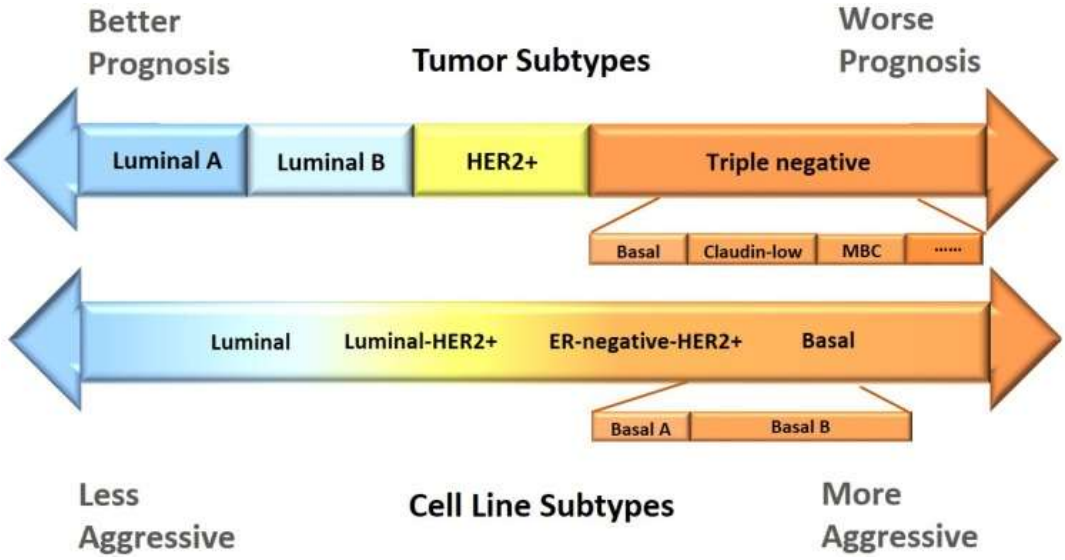


fig 1: comparison of the current subtyping schemes between breast cancer cell lines and tumors.

1.1 Luminal breast cancer cell lines

Positive ER and/or PR expression is a characteristic of luminal breast cancer cell lines, while there are a few rare exceptions, like IBEP-1 and IBEP-3 [7]. Tight cell-cell connections in luminal cell lines cause them to be less likely to migrate and, in comparison, more differentiated. This is analogous with the situation in tumor. Although the majority of studies do not further divide luminal cell lines into luminal A and B subtypes based on their HER2 status, we promote this differentiation for two reasons: first, it allows for consistent tumor subtyping classification, which makes tumor modelling easier; second, it satisfies the need for drug response assays that depend on ER and HER2 status. For example, BT474 (ER+HER2+) research has demonstrated the synergistic benefit of Herceptin and Tamoxifen in the treatment of breast cancers [8] The standard method for assessing the tamoxifen-induced cell response involves using MCF7 (ER-HER2) [9].

Hence, luminal B cells—better defined as weakly luminal cells—participate in the phenotypic attenuation of luminal A cells. Cell lines of this subtype with promise for translation in clinics have been used to successfully understand a great deal of information on luminal B malignancies [10]. For example, utilizing the ER+HER2+ cell line BT474, it is stated that the expression of quiescin-sulfhydryl oxidase 1 is related with a poor prognosis in luminal B cancers [11].

1.2. HER2 positive breast cancer cell lines

ER negative and HER2 positive cell lines have the same over-represented genomic pattern on chromosomal region 17q12, which includes genes such as HER2, GRB7, PERLD1, STARD3, and C17ORF37 [12]. These cell lines exhibit overt expression of microRNAs such as has-let-7b, has-miR-640, has-miR-200c, has-miR-378, has-miR-141, has-miR-196a, has-miR-29c, and has-miR-18a*. HER2 positive cell lines are diverse, comprising both luminal and basal traits, and they act as a link between luminal and basal cell lines. In addition to ER and HER2 expression, they are classified as luminal-ERBB2+ and ER-negative-ERBB2+ based on the expression of basal and luminal markers. Since HER2 over-expression is linked to the disruption of cell-cell junctions, cells of this subtype migrate more aggressively than luminal cells, which is consistent with their molecular characteristics [13].

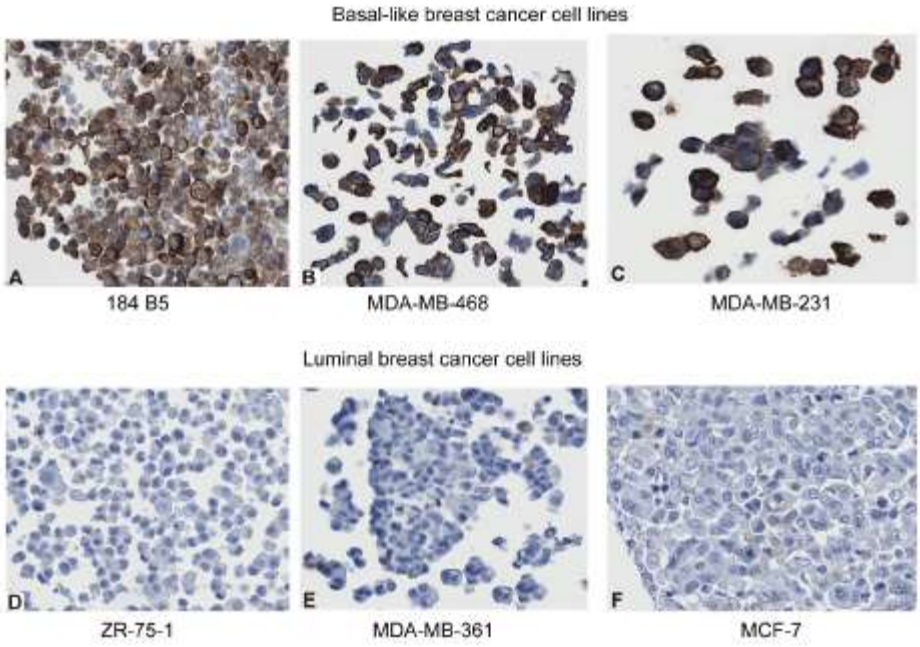


fig 2: cancer cell lines

1.3. Triple negative breast cancer cell lines

According to their scientific name, triple negative cell lines express none or very little of the three markers—ER-PR-HER2-. It is known as basal A and basal B cell lines in numerous publications and is the most diverse category overall. As basal markers such as integrins (ITGA6, ITGB4/6), cytokeratin's (KRT4/5/6A/6B/13/14/15/16/17), LAMB3, LAMC2, TRIM29, S100A2, SLPI, ANXA8, BNC1, CD10/14/58/59, MET, LYN, CD133, GABRK, VTCN1, BST2, and FABP7 are abundant in triple negative A (basal A) lines, they are referred to as basal-like [14]. Different triple negative A and B cell microRNAs are found. Specifically, the triple negative A subtype expresses hsa-miR-492, has-miR-26b, has-miR-617, and has-miT-155 openly, while the triple negative B subtype expresses hsa-miR-22, hsa-532-3p, hsa-miR-125b, hsa-miR-501-5p, and hsa-miR-155*[15]. AXL, COL3A1, COL6A1/2/3, COL8A1, MMP2/14, TIMP1, CTSC, PLAU, PLAUR, SERPINE1/2, SPARC, FN1, FBN1, HAS2, PRG1 are among the overexpressed genes linked to tumor invasive and aggressive features. Triple negative B (basal B) lines are identified as the mesenchymal cluster or normal-like/claudin-low [16]. Triple negative B cells have a more mesenchymal-like look and are more likely to be invasive, whereas triple negative A cells, a somewhat more differentiated subgroup within triple negative cell lines, can have either luminal-like or basal-like morphologies. Triple negative B cells may be utilized for claudin-low or metaplastic breast cancer modelling due to their enrichment in epithelial mesenchymal transition (EMT) and stem-cell markers. Consequently, triple negative A lines primarily resemble the core basal tumor subtype as shown by basal markers [17].

Ten triple negative cell lines—CAL148, MFM223, DU4475, KPL-3C, MA11, HCC1739, CAL120, CAL51, CAL851, and HDQ-P1—do not have direct data that can be used to further differentiate. Based on the pertinent literature, we deduce that DU4475, KPL-3C, MA11, CAL148, and MFM223 are triple negative A, and the remaining values are triple negative B. [18]. CAL148 and MFM223 are classified as the triple negative A cell line given their negative expression on vimentin. Triple negative B cells have a stemness characteristic, which is satisfied by the weakly differentiated cell line CC1739. CAL120, CAL51, CAL851, and HDQ-P1 belong to the triple negative B group because their vimentin expression is positive [19].

TABLE NO:01

Cell line	MDA-MB-231	MDA-MB-361	MDA-MB-453	BT-474	SK-BR-3
Source	Pleural effusion	Brain metastasis	Pericardial effusion	Invasive ductal carcinoma	Pleural effusion metastasis
Karyotype	Hypo-triploid	Hyper-diploid	Tetraploid	Hyper-triploid	Hyper-triploid
Type	Basal B	Luminal	Luminal	Luminal	Luminal
Hormone status	Triple Negative	Triple Positive	Negative ER/PR	Triple Positive	Negative ER

1.4.MCF-7 breast cancer cell line

History:

The name MCF-7 comes from the Michigan Cancer Foundation, where it was founded in 1973 by Dr. Soule and others. The cells were isolated from a 69-year-old woman's pleural effusion who had metastatic illness [20]. Hormone therapy and radiation treatment managed local recurrences for three years. Before beginning primary cell culture, the patient had undergone a radical mastectomy of her right breast for a benign tumor seven years prior, and a second mastectomy of her left breast for a malignant adenocarcinoma four years later. A pivotal discovery for breast cancer was the description of ER in the MCF-7 cells in 1973. Subsequently, in 1975, it was shown that tamoxifen, an anti-estrogen, could stop MCF-7 cell growth, but that estrogen could also reverse this suppression. The main goal of laboratory research in the 1970s and early 1980s was to demonstrate that estrogen directly encouraged tumor growth, despite the intriguing results with antioestrogens [21].

Characterization

MCF-7 is a widely utilized cell line for breast cancer that has been reproduced for many years by several organizations [22]. It shows proven to be a good model cell line for global research on breast cancer, including studies including anticancer medications [23]. MCF-7 is a non-invasive, less aggressive cell line that is typically thought to have a minimal propensity for metastatic spread [24]. MCF-7 cells have been employed in most studies on acquired anti-estrogen medication resistance and are widely used in research for ERpositive breast cancer cell assays. Because MCF-7 cells are easily cultivated and maintain ER expression after receiving such targeted therapy, they are a good choice for investigations investigating the resistance of antihormone therapy. Populations of MCF-7 cells that have been adapted to different anti-hormone settings have been produced in order to study the characteristics of acquired antihormone-resistant breast cancer cells [25]. Divergence occurs between several MCF-7 deviations at the genomic and RNA expression levels [26].

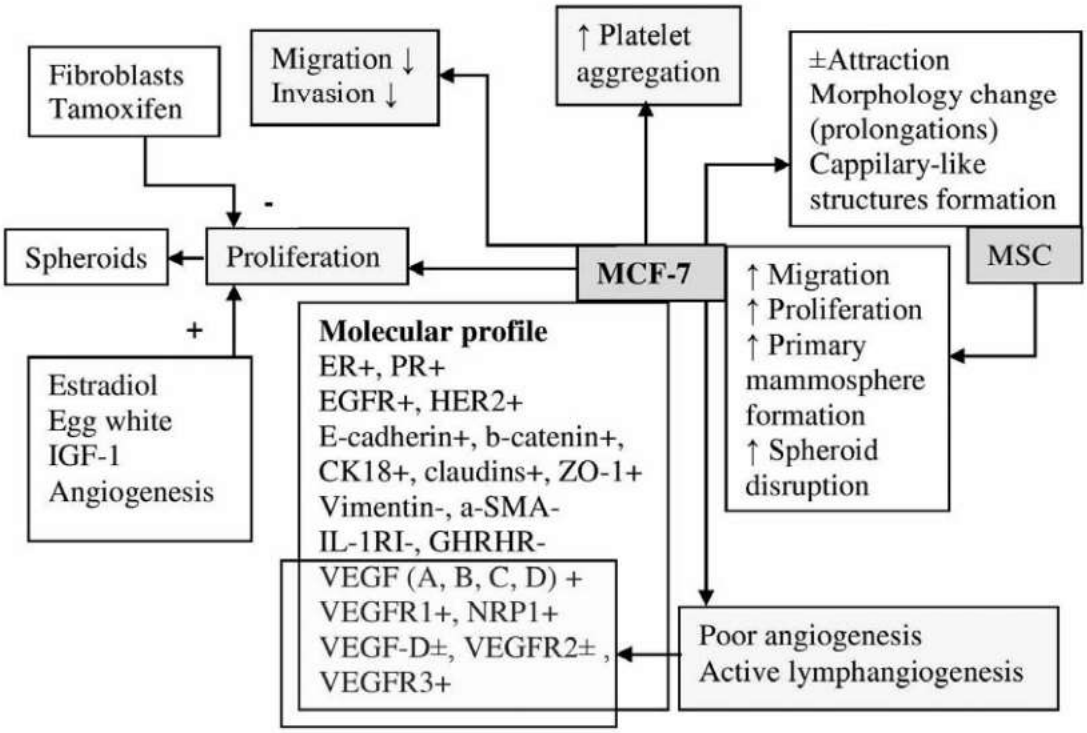


fig 3: the molecular profile and properties of mcf-7, as well as the interaction between mcf-7 and the mesenchymal stem cells [27].

Molecular profile

Estrogen (E2) is sensitive to MCF-7 breast cancer cells, and they require E2 to multiply [28]. While some scholars contend that the parental line's ER expression is weaker than that of the tamoxifen-resistant sub-lines, other studies have shown that MCF-7 contains a sizable number of 17β-estradiol receptor [29]. The expression of ER increases in MCF-7 cells when estrogens are not present. MCF-7 cells respond differently to short-term (less than six months) versus long-term (more than six months) estrogen deprivation. When estrogen is removed, there is a brief decrease in proliferation that occurs around a month later, suggesting that MCF-7 has not yet developed compensatory or adaptive growth mechanisms [30]. Along with ER and PR, plasma membrane-associated growth factor receptors also regulate the proliferation of breast cancer cells. The human epidermal growth factor receptor-2 (HER2), which is present in MCF-7 cells, and the epidermal growth factor receptor (EGFR), which is activated by epidermal growth factor (EGF), are two members of this broad family that are particularly significant [31]. MCF-7 cells display characteristics of differentiated mammary epithelium: they are negative for mesenchymal markers like vimentin and smooth muscle actin (SMA), and positive for epithelial markers like E-cadherin, β-catenin, and cytokeratin 18 (CK18) [32].

2.THYRIOD CANCER CELL LINES

Introduction:

The most popular models for thyroid cancer research are human thyroid cancer cell lines. They need to be used with a thorough understanding of their properties. These in vitro cell lines are from human thyroid cancers that have both differentiated and dedifferentiated in vivo. Both in vitro and in vivo models are used in the experimental research of human malignancies. Human cancer cell lines are often employed as one of the many potential experimental models [33]. The most common endocrine cancer is thyroid cancer. Thyroid carcinomas come in different forms, but the most common ones are thyrocyte-derived ones, which include papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), anaplastic thyroid carcinoma (ATC), and poorly differentiated thyroid cancer, which is a form in between PTC/FTC and ATC. A group of mutations that cause enhanced cellular proliferation and dedifferentiation characterize every type [34]. The most common kind of thyroid cancer in humans is PTC. BRAF point mutations, which occur in 40–60% of PTC cases, and RET/PTC rearrangements, which occur in 20% of cases, are the most common genetic abnormalities detected in PTC. The predominant BRAF mutation takes place in the serine/threonine kinase domain (V600E) and results in the protein's constitutive kinase activity [35].

Materials & Methods

The cell lines used in this investigation for thyroid cancer were created in our labs, obtained from repositories or, where feasible, straight from the source. We looked at sixty cell lines, excluding ML-1 and THJ-11T. THJ-11T produced sequencing data of poor quality.
The ML-1 gene expression profile from the CCLE was used in these investigations as our examination of two separate vials of the ML-1 cell line kept in our labs revealed signs of contamination from BHT-101 cells. We offer data on 58 cell lines for mutational investigations. Every cell line was cultivated in the prescribed media and kept at 37°C with 5% CO2 in a humidified environment [36].

RNA Extraction

The cells grown in 10 cm dishes were given 2 ml of Triazole Reagent (Ambion, Austin, USA) after the growth media was removed. The manufacturer's instructions for extracting total RNA were followed by purification using miRNAs columns (QIAGEN, Hilden, Germany). RNA was measured using spectrophotometry, and automated gel electrophoresis (Experion, Bio-Rad, Hercules, USA) was used to confirm the integrity of the material.

RT-PCR

RT-PCR Following a DNase treatment using the DNase I Amplification Grade kit (Invitrogen, Carlsbad, USA), 1µg of total RNA was used for reverse transcription with the aid of reverse transcriptase (Superscript II RNase H Reverse Transcriptase kit, Invitrogen) and hexamers (3.6µg/µl) (Roche, Basel, Switzerland). Using the recombining Taq DNA polymerase kit (Invitrogen), PCR procedures were carried out. 5µl 10X PCR buffer, 1.5µl MgCl2 (50 mM), 1µl dNTP mix (10 mM, Invitrogen), 1µl each of the forward and reverse primers (10µM), 0.4µl Taq DNA polymerase (5U/µl), 2µl of the RT reaction's DNA, and 38.1µl of water were used for each PCR reaction [37].

Correlation of mrna expression between in vitro human thyroid cancer cell lines and in vivo human thyroid tumor

Using R (version 2.14.1), Spearman's correlation coefficients were calculated. Using a Mann–Whitney U-test, correlation coefficients between the gene expression profiles of ATC and thyroid cancer cell lines and those of PTC or FTC and thyroid cancer cell lines were evaluated based on the merged file. This evaluated the degree of mRNA expression profile similarity between ATC and cell lines compared to FTC or PTC and cell lines. These coefficients were compared among samples that were hybridized on the same microarray platform. Based on those Spearman correlation coefficients, 2D-multidimensional scaling was shown using the R function isoMDS from the MASS v.7.3–18 package.

Analysis of the genes commonly and oppositely modulated in act in vivo and cancer cell lines

Genes regulated in ATC compared to normal tissues and in cell lines compared to primary cultured normal thyrocytes were identified using scores and q-values calculated using a slightly modified version of Rank Products (Breitling et al., 2004). Regardless of any technological variations, such as from different laboratories or microarray platforms, Rank Products is a non-parametric method that enables the investigation of differential expression and the integration of data from various datasets. Demonstrated to perform better than SAM
[38]. It outperforms t-test based statistics in terms of power even with less samples. The genes identified from ATC and cell lines have a q-value of less than 0.05. The following genes were retained and displayed in a heatmap using the R library since their regulation was consistent in both ATC and cell lines: gplots version 2.10.1. Genes that exhibited notable differential regulation between ATC and cell lines were further examined in more detail.

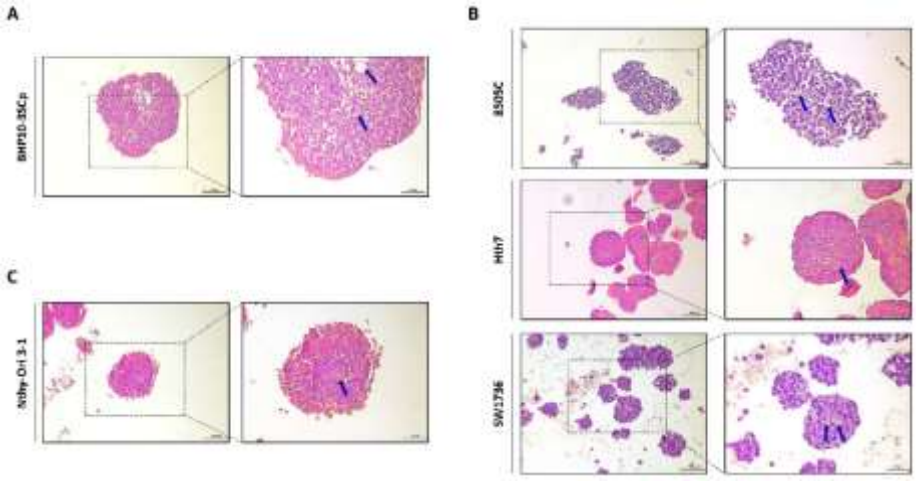
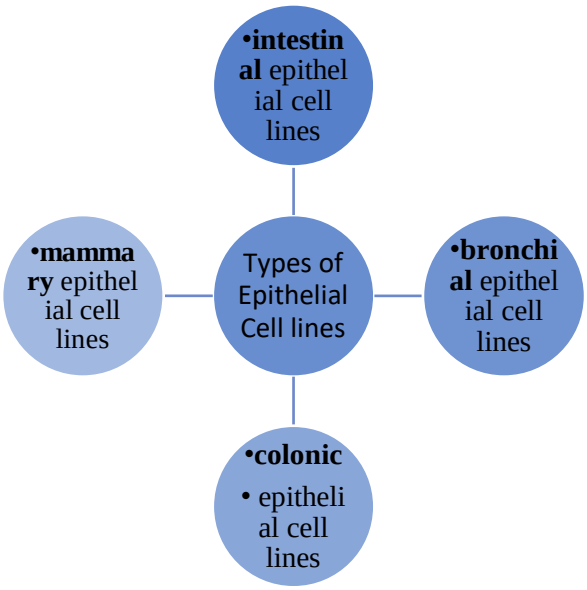


fig 4: thyroid cancer cell line

3.EPITHELIAL CELL LINES

Appropriate in vitro models are used by the pharmaceutical industry to assess medication absorption and metabolism. There isn't a commonly used cell culture model of the airway epithelium at the moment, despite growing interest in drug administration through the lungs. The airway epithelium, airway epithelial cell culture, and the requirement for cell lines that can mimic the airway epithelium are all covered in this article. This review focuses on the recent uses of three of the most promising human bronchial cell lines—16HBE14o–, Calu-3, and BEAS-2B—for the investigation of drug transport, drug metabolism, and gene delivery [39].

TYPES OF EPITEHLIAL CELL LINES



3.1. Intestinal Epithelial cell line

Studies on the form and function of intestinal epithelium may benefit from the use of confluent monolayers of epithelial cell lines of intestinal origin. T84 line cells seem to be a good model to employ for studying C1-secretion. We now outline this cell line's fine structure, the structural processes involved in the generation of monolayers, and the effects of permeable versus impermeable substrates and plastic versus collagen substrates on the structural growth of these monolayers. T84 cells cover the filter surface when grown on collagen-coated filters at confluency, and in 18 hours, they deposit material that resembles basement membranes. These "monolayers" exhibit a multifocal pattern where regions consisting of a single layer of cells alternate with sections containing several cell layers. Cell polarization is modest at this developmental stage, ultrastructural discontinuities that confer low transepithelia1 resistance are present, and the morphologic characteristics have resemblance to the stratified intestinal epithelium seen in fatal development. True monolayers form on collagen-coated filters after 5 days of plating. These monolayers are composed of highly polarized columnar cells that resemble intestinal crypt cells structurally and have a high transepithelia1 resistance. Although cells plated on plastic substrates can also

produce material resembling basement membranes quickly, they cannot initiate the rapid spreading of their cells over the substrate surface under these conditions, in contrast to cells plated on collagen-coated filters. Because of this, after five days, monolayers plated on plastic are less homogeneous than those plated on collagen-coated filters. Additionally, they show many macromolecule leaks and areas with a high concentration of unpolarized, flattened cells. Studies utilizing collagen-coated filters created impermeant with glass backing indicate that baso1ateta1 membranes' increased access to nutritional medium when grown on permeable supports is not the only reason for this substrate's enhancing effect on monolayer structural development. This work highlights the significance of substrate selection while defining the structure and developmental history of this practical model epithelium [40].

It has been observed that a number of human colon cancer cell lines, including Caco-2, T-84, and HT-29, have varied degrees of enterocytic differentiation when grown in culture [41]. The intricate geometry and diverse cellular makeup of the intestinal epithelium frequently provide challenges to the analysis of its function [42]. For example, any species of ion's net trans epithelial movement is the average of several transport events carried out by different cell types and regulated by neuronal and lamina propria components. Thus, it would be a significant methodologic advancement to be able to analyse transport across cultivated monolayers made up of a single species of epithelial cells. The best source of monolayers would be initial cultures of absorptive cells, undifferentiated crypt cells, goblet cells, etc. from mammals that are typically functioning. There have been some overall advancements made in achieving this goal [43].

To this objective, the utilization of immortal cell lines from intestinal cancers would be a reasonable alternative. However, these cell lines must have structural and functional similarities to specific intestinal epithelial cell types in order to be valuable models for research on intestinal function [44].

It has recently been demonstrated that the T84 cell line, which was obtained from a lung metastasis of a human colonic cancer, may develop as a monolayer of low cuboidal epithelium on plastic. T 84 cells generate confluent mono layers that can be mounted in Using-type chambers when they are plated at high density on collagen-coated filters. In these conditions, these monolayers demonstrate a high electrical resistance and the capacity to secrete Cl- in response to a range of secretagogues [45]. The cellular replication and differentiation of the intestinal epithelium have geographical boundaries that are distinguished by unique phenotypes [46]. Villi that extend into the lumen make up the small intestinal epithelium. Terminally developed mucous and absorptive cells make up the majority of the epithelium, and replicating cells, including possible stem cells, exist in crypts at the base of villi. The replication units of villi are made out of stem cells [47]. The offspring cells differentiate into mucous goblet, absorptive, and endocrine cells as they mostly move upward forming villi. The Paneth cells, which are also thought to have stem cell ancestry, move below the replicative units and release growth factors and antimicrobial peptides in the form of lysozymes. With the exception of lacking villi and Paneth cells, the colonic mucosa resembles that of the small intestine in most aspects [48].

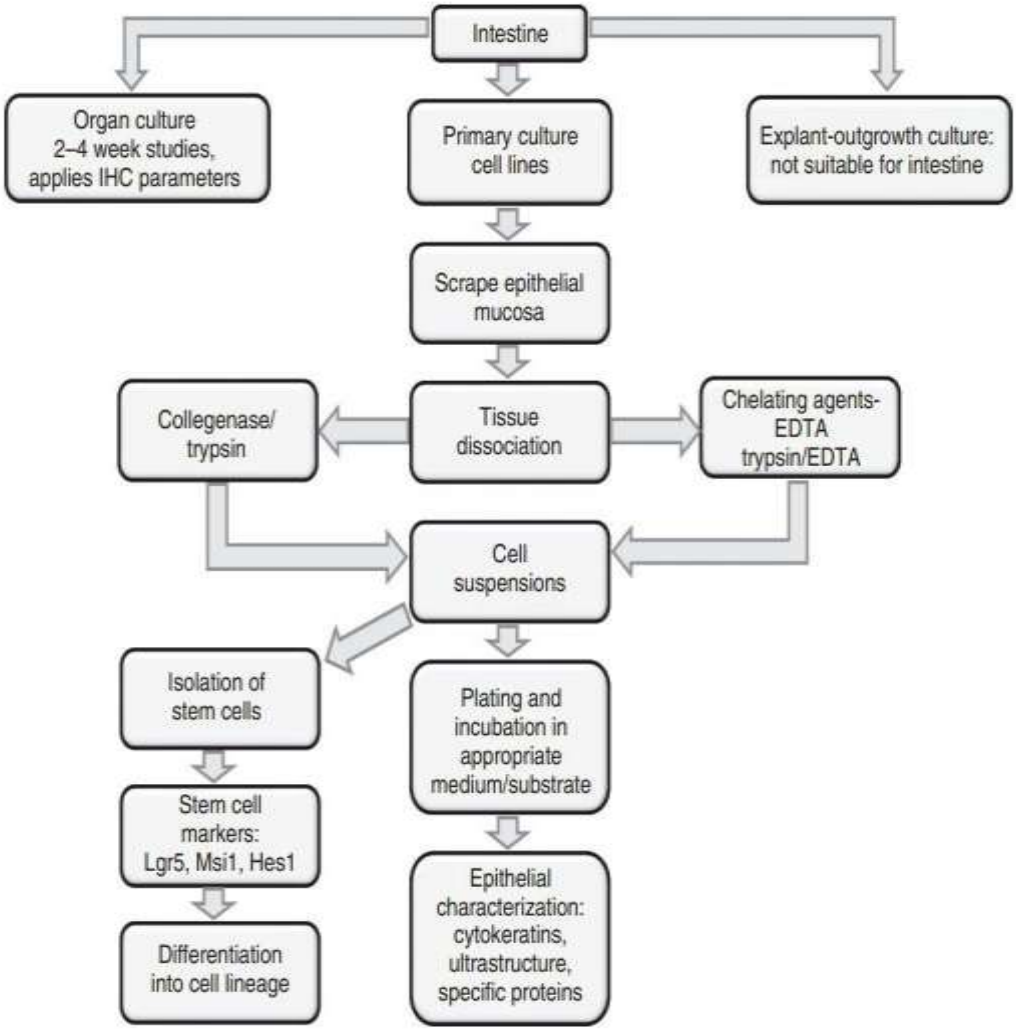


fig 5: in vitro methods to investigate intestinal epithelial cells [*49]*

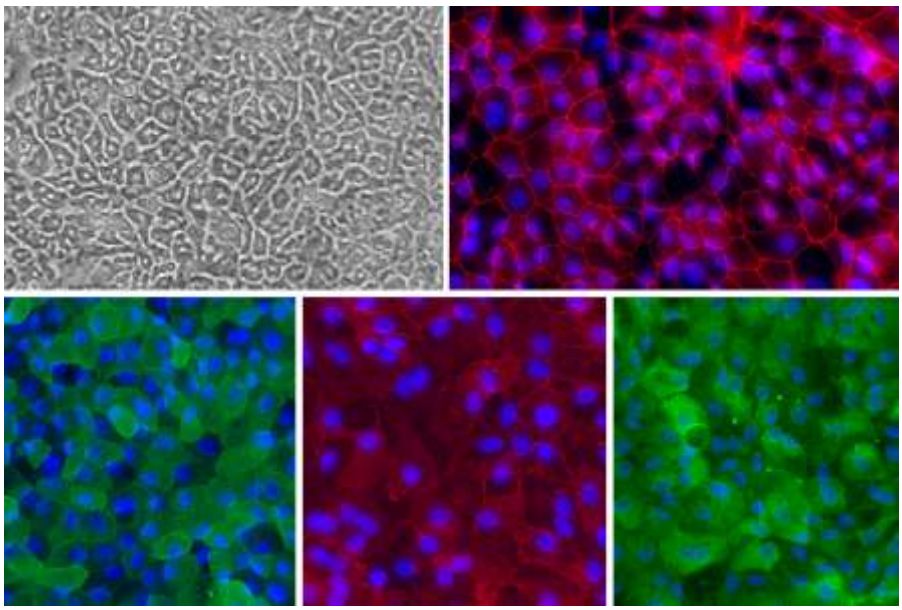


fig 6: intestinal epithelial cell lines

3.2. Bronchial Epithelial Cell Lines

Human bronchial epithelial (hBE) cells often lose their ability to differentiate when they immortalize. The protooncogene Bmi-1 preserves stem cells, and when it is expressed, cell lines that mimic normal cell structure and function are produced. We treated three non-cystic fibrosis (CF) and three F508 homozygous CF primary bronchial cell preparations with Bmi-1 and the catalytic subunit of telomerase (hTERT). While not as significantly as viral oncogenes, this treatment prolonged cell life span, and at passages 14 and 15, the newly created cell lines

possessed a diploid karyotype. Variable transepithelial resistances, ranging from 200 to 1,200 cm2, were found using Ussingchamber analysis. Forskolin enhanced short-circuit currents in the non-CF cell lines, but CFTR(inh)-172 suppressed them at levels mostly corresponding to early passage primary cells. Forskolin-stimulated current was absent from CF cell lines, and the CTR (inh)-172 response was negligible. There were amiloride-inhabitable and UTP-stimulated currents, albeit they were not as strong as they were in primordial cells. With noticeable apical membrane polarization, few apoptotic bodies, a large number of mucous secretory cells, and sporadic ciliated cells, the cells had a pseudostratified appearance. Comparable amounts of IL-8 were secreted at baseline by CF and non-CF cell lines, and they also increased in response to IL-1, TNF, and the Toll-like receptor 2 agonist Pam3Cys. Bmi-1/hTERT airway epithelial cell lines will be helpful for various research routes and will assist bridge gaps now impeding CF research and therapy development, despite their slower growth potential and more stringent growth requirements than viral oncogene transformed cells.

An important environmental interface that is involved in a number of respiratory disorders is THE AIRWAY EPITHELIUM. This system's in vitro models are essential for study and development. Animal and human respiratory epithelial cells have been cultivated, either as protease-dissociated cell suspensions or as proliferating from tissue fragments that were transplanted [50]. When exposed to chemical and physical cytotoxic substances, human airway epithelia frequently experience hyperplasia, squamous metaplasia, and dysplasia. Strict interregulation of cell replication and regulatory mechanisms for squamous and mucoepidermoid differentiation are required for these processes. A significant probability of neoplastic transformation is positively correlated with deviations in these processes. Investigations into these phenomena can be facilitated by using replicative cultures of normal human bronchial epithelial (NHBE) cells. This chapter examines popular techniques for creating these cultures and provides a quick overview of possible changes [51].

Together, the secretions of various secretory cells of the mucosa lining the airway maintain the inspired air moist and free of dust, germs, and gases that may be adsorbed. In addition to safeguarding the lung's respiratory zone, mucus-secreting cells function as pluripotent stem cells in fetuses and in adults after mucosal damage. The mucous and serous cells of the surface and glandular epithelium, the non-ciliated bronchiolar (Clara) cell, and the less common dense-core granulated (neuroendocrine) cell are among the different types of secretory cells. The latter group is the first to differentiate, starting about week 10 of gestation; mucus-secreting cells appear around week 13, when mature ciliated cells are already present, and Clara cells start to mature around week 19 of human development. The second trimester of fatal life is characterized by changes in the number of secretory cells and the chemical makeup of their secretions, which are comparable to adult chronic bronchitis. However, in hypersecretory illness, the location and magnitude of the primary alteration seem unsuitable for lung defines [52].

Much less is known about the progenitor cells from the human lung, despite the fact that the adult mouse lung uses several compartmentally restricted progenitor cells during homeostasis and repair. The anatomical variations between species make it difficult to apply the mouse stem cell model to human patients. Here, we demonstrate that human bronchial epithelial cells (HBECs) exhibit traits of lung multipotent stem cells. When tested experimentally in cell culture, these HBECs exhibit markers suggestive of many epithelial types of the adult lung. Slight alterations in the microenvironment produce distinct reactions in three distinct three-dimensional (3D) systems, one of which is the capacity of HBECs to differentiate into various types of peripheral and central lung cells. These novel discoveries suggest that a multipotent progenitor cell whose potential for differentiation is largely determined by the microenvironment exists in the adult human lung. The HBEC system is crucial for investigating alterations associated with human lung disorders, such as lung cancer, as well as for comprehending the mechanisms underlying particular cell lineage differentiation [53].

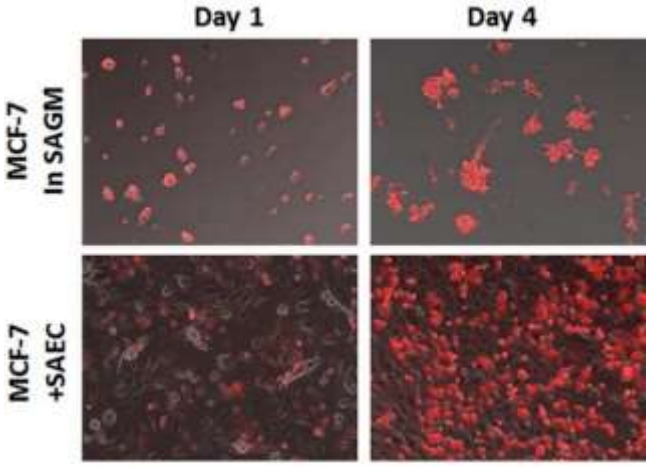


fig 7: human bronchial epithelial cell lines

3.3. colonic epithelial cell lines

Colon cancer risk is lowered by physical activity but increased by obesity. Physical activity lowers plasma levels of leptin, which rise in direct proportion to obesity. Since leptin is a growth factor for multiple cell types, it could offer a scientific explanation for the risk variables linked to the observed epidemiology. This study aimed to determine if leptin stimulates colonic epithelial cells to proliferate [54]. The availability of normal cell lines that are simple to develop in vitro will greatly aid in the advancement of knowledge regarding this significant illness [55]. Understanding the biological basis of carcinogenesis and other colon illnesses requires an understanding of normal colonic function. However, the unavailability of long-term cultures of normal human colonic epithelial cells has hindered research on colonic pathophysiology. The current work set out to create techniques for separating live human colonic epithelial cells in order to create colonic epithelial cell lines that are not altered. Normal human colons that underwent surgery were used to isolate human colonic epithelial cells. We discovered that using a brief enzymatic digestion consistently produced more viable human colonic epithelial cells (>90%). These separated colonocytes were cultured using various medium formulations on feeder layers, plastic, or filters covered with collagen. To create long-term cultures of nontrans formed human colonic epithelial cells, the colonocytes from the original primary cultures that seemed the most "epithelial" were cloned and passaged [56].

Because normal human large intestinal epithelial cells (NHLIEC) have not been cultured for an extended period of time, research on normal cellular activity and the knowledge of cellular mechanisms of carcinogenesis and other illnesses of the large intestine have been restricted. We have successfully isolated a sufficient number of live NHLIEC and cultured them in vitro for up to five months using the epithelia from surgically removed human colons. In order to separate the cells, 0.01 mg/ml trypsin, 0.2 mg/ml collagenase + 0.1 mM EGTA, or 0.1 mg/ml trypsin + 0.1 mM EGTA were applied to human large intestine mucosal pieces (1 mm2). Up to 84% of the cells per gram of tissue were alive when using the Stomacher blender in conjunction with low proteolytic enzyme doses, as compared to other procedures. NHLIEC were cultivated in serum-free KGM, MEM containing 5% serum, and CMRL-1066 for both primary and serial passages. Insulin, hydrocortisone, epithelial growth factor, and bovine pituitary extract were added to each of these medium as supplements. It was discovered that the ideal medium for NHLIEC was CMRL-1066. By permitting the cells to stick to the culture vessel for a brief period of time and adding 25 U/ml collagenase to the culture media during the first subculture treatment, contaminating fibroblasts were selectively eliminated. Immunoreactivity to cytokeratin, positive mucin cytochemistry, and morphological criteria (light, phase contrast, and electron microscopy) all supported the established cells' epithelial origin and secretory function. We suggest that this methodology be used to the long-term culture and upkeep of NHLIEC, as it could be a useful model for a range of studies [57].

This work aimed to create an in vitro model of colorectal carcinogenesis tumor progression by converting the premalignant human colonic PC/AA adenoma cell line to the malignant phenotype. From the diploid PC/AA adenoma cell line, a rare clonogenic variation known as AA/C1 [colony-forming efficiency (CFE) on plastic of 1.05%] was discovered. The aneuploid cell line AA/C1/SB was created after 14 days of treatment with 1 mM sodium butyrate. This cell line displayed a higher CFE on plastic (6.13%), but it was still anchorage dependent and nontumorigenic. Following the carcinogen N-methyl-N′-nitro-N-nitrosoguanidine exposure of these AA/C1/SB cells, an anchorage-independent cell line (AA/C1/SB10) was identified. The CFE in agarose of AA/C1/SB10 grew to 17.3% after continuous in vitro passage, and the cells become tumorigenic, causing adenocarcinomas in athymic nude mice. Common chromosomal abnormalities in AA/C1, AA/C1/SB, and AA/C1/SB10 cell lines include monosomy for chromosome 18 and pericentric inversion of chromosome 1, which has a portion of the short arm deleted. The adenoma to carcinoma sequence in the human colon is first experimentally demonstrated by this in vitro process, and cytogenetic data indicates that it may be important to in vivo carcinogenesis [58].

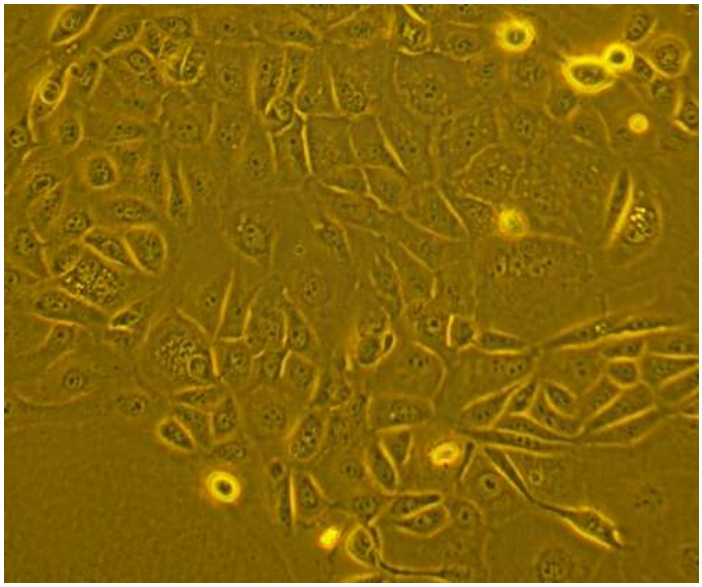


fig 8: human colon epithelial cell line

3.4. Mammary epithelial cell lines

The branched duct network that makes up the resting gland's cellular architecture is where the mammary gland's growth and development during pregnancy and lactation begin. The expansion of ducts and lobule alveoli within the mammary fat pad characterizes the majority of growth, which happens during pregnancy. As parturition approaches, cells of the future secretory epithelium transform into secretory cells [59]. When the lactation stimulation stops, mice' mammary epithelial or alveolar cells experience severe cell loss and degeneration as the gland involutes into a primary ductal shape [60]. In contrast, there is very little cell loss in cattle during post lactational involution [61]. This illustration highlights the risk associated with extrapolating mouse model behaviour to cow mammary gland behaviour. There are three immortal cell lines of cow mammary epithelial cells. A spontaneously immortalized cell line called HH2A generates a growth inhibitor derived from mammaries [62]. Most of the research done on the BMEC + H line has focused on cytokeratin's and desmosome plaque proteins [63]. For any of the aforementioned cell lines, casein synthesis has not been documented. At first, it was believed that the MAC-T cell line could synthesize casein and undergo differentiation [64]. But these coils seem phenotypically unstable in our hands, and casein 1To whom letters should be sent. Expressions are quite unpredictable [65]. Moreover, the known modulator of mammary function, epidermal growth factor (EGF), had little effect on MAC-T cells. A dependable bovine mammary epithelial cell line that maintains the characteristics unique to mammary epithelium, such as EGF responsiveness, is still lacking. With the help of this cell line, researchers would have more chances to investigate the controls over the growth and differentiation of bovine mammary epithelial cells. We show that primary bovine mammary epithelial cells are established in culture upon expression of the SV40 big T-antigen. Through microinjection, the SV40 big T-antigen sequence-carrying plasmid was delivered into cells. The morphogenetic behaviour, long-term proliferation potential, and differentiation properties of the generated cell lines were analysed [66].

Human mammary epithelial cells (HMECs) do not spontaneously multiply in culture and have a limited lifespan. The ability of cells to transcend the senescence checkpoints that limit their replicative life span and to reproduce endlessly—a process known as immortalization—is essential to malignant transformation. By subjecting HMECs to radiation or chemicals, or by inducing overexpression of specific viral oncogenes or cellular genes, it is possible to render them immortal. However, the expression of the high-risk HPV oncogenes E6 and E7 continues to be the most effective and repeatable model of HMEC immortalization. The function of tumor suppressor proteins (pRb and p53), telomerase, p14ARF, small G proteins Rap, Rho, and Ras, and inhibitors of cyclin-dependent kinases (p16INK4a, p21, p27, and p57) in the immortalization and transformation of HMECs has been elucidated using cell culture models. The existence of many epithelial cell subtypes in normal breast tissue, each with a unique pattern of vulnerability to oncogenesis, has also been demonstrated using these cell culture models. These results imply that different mammary cell subtypes may be antecedents of unique subtypes of breast cancers when combined with data from distinct molecular profiles of primary breast cancers. Raf, phosphatidylinositol 3-kinase, hTERT (the catalytic component of human telomerase), SV40 big T and small t, and Ral-GEFs (Ral guanine nucleotide exchange factors) must all be expressed for HMECs to fully undergo oncogenic transformation in culture. However, these altered cells usually result in poorly differentiated carcinomas rather than

adenocarcinomas when inserted into naked mice. However, adenocarcinomas are developed in transgenic mice models employing ErbB2/neu, Ras, Myc, SV40 T, or polyomavirus T. This suggests that the pathological type of breast tumor may be determined by the normal cell subtype of the parents. The discovery of putative stem cells has been facilitated by the availability of three-dimensional and mammosphere models; however, additional research is required to clarify their biologic role and potential as precursor cells for different types of breast cancer. Understanding the nature of breast cancer diversity and creating customized treatments should be made easier with the combined application of transformation techniques in cell culture and animal models as well as the genetic identification of human breast cancer subtypes [67].

Using particular genes to turn primary human mammary epithelial cells (HMECs) into carcinoma cells is one method of determining the genetic and metabolic changes necessary for this process. Here, we demonstrate that when three genes encoding the telomerase catalytic subunit, the SV40 large-T antigen, and an H-Ras oncoprotein are introduced into primary HMECs, the cells develop tumor when transplanted subcutaneously or into the mammary glands of immunocompromised mice. The degree of ras oncogene expression determined how tumorigenic these changed cells were. It's interesting to note that amplifications of the c-myc oncogene during the in vitro cell growth were linked to the transformation of HMECs but not two other human cell types. The altered HMECs gave rise to poorly differentiated carcinomas that permeated the surrounding tissue to create tumor. Tumor developed after just half of the subcutaneous injections of these cells, with an average latency of 7.5 weeks. The stromal microenvironment has a major impact on tumorigenicity, as evidenced by the fact that mixing epithelial tumor cells with Matrigel or primary human mammary fibroblasts significantly increased the efficiency of tumor development and lowered the latency of tumor formation [68].

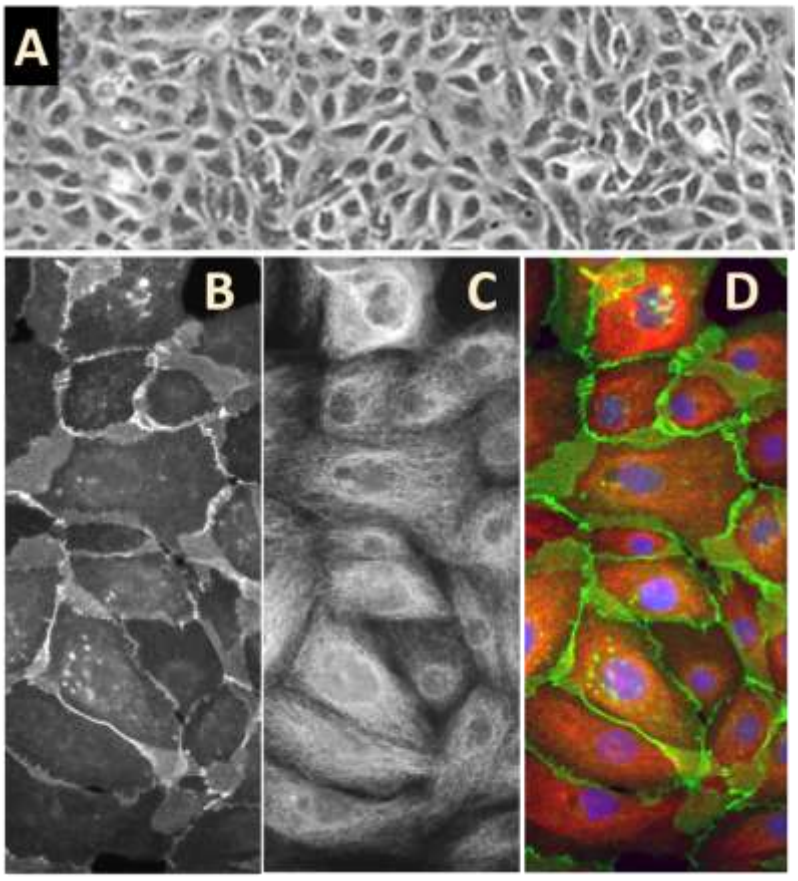


fig 9: human mammary epithelial cell lines

CONCLUSION

This review successfully explains the different types of cell line that are used in the in vitro study of the novel drug. Cell lines like breast cancer cell line, thyroid cancer cell line, epithelial cell line and its types. Thus, this study helps in the in vitro study of drug on different cell lines.

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