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Review

The Story of MCF-7 Breast Cancer Cell Line: 40 years of Experience in Research

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Abstract. *Breast cancer is the most frequent malignancy in females. Due to its major impact on population, this disease represents a critical public health problem that requires further research at the molecular level in order to define its prognosis and specific treatment. Basic research is required to accomplish this task and this involves cell lines as they can be widely used in many aspects of laboratory research and, particularly, as in vitro models in cancer research. MCF-7 is a commonly used breast cancer cell line, that has been promoted for more than 40 years by multiple research groups but its characteristics have never been gathered in a consistent review article. The current paper provides a broad description of the MCF-7 cell line, including the molecular profile, proliferation, migration, invasion, spheroid formation, its involvement in angiogenesis and lymphangiogenesis and its interaction with the mesenchymal stem cells.*

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths; thus, research in this field is important to overcome both economical and psychological burden (1). In recent years it has become clear that breast cancer does not represent a single disease but rather a number of molecularly-distinct tumors arising from the epithelial cells of the breast (2).

Cell lines seem to be a key element for the molecular diagnosis in breast cancer as they can be widely used in many aspects of laboratory research and, particularly, as *in vitro* models in cancer research (3). As for breast cancer, MCF-7 cells represent a very important candidate as they are used ubiquitously in research for estrogen receptor (ER)-positive breast cancer cell experiments and many sub-clones, which have been established, represent different classes of ER-positive tumors with varying nuclear receptor expression levels (4).

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History

Established in 1973 by Dr. Soule and colleagues at the Michigan Cancer Foundation, where from their name derives, MCF-7 cells were isolated from the pleural effusion of a 69-year-old woman with metastatic disease (5). The patient had undergone a mastectomy of her right breast for a benign tumor 7 years before primary culture of cells was started and a consecutive radical mastectomy of her left breast for a malignant adenocarcinoma 4 years later (6). Local recurrences were controlled for 3 years with radiotherapy and hormone therapy (5). In the days before tamoxifen, the patient was probably treated with high doses of the synthetic estrogen diethylstilbestrol and the disease was controlled for three times longer than expected proving that the tumor was hormone-responsive. Two months after widespread nodular recurrences occurred, in June of 1970, samples were taken from a pleural effusion for laboratory studies (6).

A pivotal discovery for breast cancer was the description of ER in the MCF-7 cells in 1973 (5). Further on, in 1975, it was demonstrated that the anti-estrogens tamoxifen inhibited the growth of MCF-7 cells but the inhibition could be reversed by estrogen. Despite the interesting findings with antiestrogens, the central focus of laboratory research in the 1970s and early 1980s was to prove that estrogen directly stimulated tumor growth (6).

Characterization

MCF-7 is a commonly used breast cancer cell line, that has been propagated for many years by multiple groups (7). It proves to be a suitable model cell line for breast cancer investigations worldwide, including those regarding anticancer

drugs (8). With time, MCF-7 has produced more data of practical knowledge for patient care than any other breast cancer cell line (4) (Table I). It is ER-positive and progesterone receptor (PR)-positive (8) and belongs to the luminal A molecular subtype (2). MCF-7 is a poorly-aggressive and non-invasive cell line (9), normally being considered to have low metastatic potential (8).

The human breast MCF-7 line, although often treated as a single entity, comprises of large number of individual phenotypes most of which constitute only small proportions of the total population. These phenotypes differ in gene expression profile, receptor expression and signaling pathway. Despite differences in the proliferation rate of individual phenotypes, a balance of multiple phenotypes is somehow maintained during progressive culturing of the line, perhaps by some type of signaling cooperation. The small sub-lines existing in the parental line can be expanded under appropriate selective conditions. The time scale of the *in vitro* selection process (6 months or more) is consistent with the long period of time that occurs clinically in the development of resistance to anti-estrogen therapy or aromatase inhibitors in breast cancer patients. However, a critical question with regard to therapy is whether the emerging sub-lines express altered receptors and associated signaling pathways (7).

Quite early in the history of MCF-7 cells reports on clonal variations were made in the literature. MCF-7 cells present extensive aneuploidy with important variations in chromosome numbers ranging from 60 to 140 according to the variant examined. Other cytogenetic differences concerned the presence or absence of specific marker chromosomes. The available data suggested an elevated level of genetic instability in MCF-7 cells. The karyotypic differences could reflect changes in selective pressure due to different culture conditions. MCF-7 cells contain a fraction of stem cells able to generate clonal variability. This was proposed as an explanation for the heterogeneity of this cell line and as a model for breast tumor heterogeneity. Different MCF-7 variants undergo divergence at both the genomic and the RNA expression levels (10).

MCF-7 cells are used ubiquitously in research for ER-positive breast cancer cell experiments, with the majority of the investigations into acquired anti-estrogen drug resistance having utilized them. MCF-7 cells are well-suited for anti-hormone therapy resistance studies since they are easily cultured and retain ER expression when they were treated with such targeted-therapy. To investigate the properties of acquired antihormone-resistant breast cancer cells, populations of MCF-7 cells -adapted to various anti-hormone environments- have been created (4).

In vitro, MCF-7 models eventually evolved one step further toward clinical practice when they were adapted to *in vivo* models, which mirror more closely clinical care. *In vivo* models create a new dimension to assess the importance of the interaction between cancer cells, angiogenesis, cellular metabolism and respiration, processes that cannot be properly evaluated in cell culture (4).

Cell Culture

MCF-7 human breast cancer cells may be seeded in T75 flasks at 1×10^6 cells/flask in low glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 2 mM glutamine, 0.01 mg/ml insulin and 1% penicillin/streptomycin mix and need to be incubated at 37˚C in an atmosphere of 5% CO_2. The medium renewal has to be performed 2 times per week, while cells should weekly be passaged at a sub-cultivation ratio of 1:3 (11). An alternative cell culture medium would be DMEM supplemented with antibiotics/antimycotics and 10% FBS (12).

Molecular Profile

MCF-7 breast cancer cells are estrogen (E2)-sensitive cells and depend on E2 in order to proliferate (13). They express high levels of *ERα* transcripts but low levels of *ERβ* (14). Although some authors suggest that the ER expression is weak in the parental line when compared to that of the tamoxifen-resistant sub-lines (7), others have demonstrated that MCF-7 contain significant amounts of 17β-estradiol receptor (15). On the other hand, the expression of PR is strong in the parental line but weak or even absent in the tamoxifen-resistant sub-lines (7). MCF-7 cells increase expression of ER in the absence of estrogens. Short-term estrogen deprivation causes distinct responses of MCF-7 cells in comparison to long-term (over six months) estrogen deprivation. A reduced proliferation rate lasts for about a month after estrogen removal indicating that, during this period, MCF-7 have not found adaptive or compensatory growth mechanisms yet (4).

The differences in growth, as a response to E2 treatment of the MCF-7 strains, are not caused by differences in ER expression level or functionality. E2 responsiveness of MCF-7 cells seems to be dependent by the secretion of an autocrine factor activating the insulin-like growth factor type I receptor (IGF-IR) (16). There also exists evidence regarding a role for IGF-1 signaling in the regulation of miRNAs in MCF-7 cell line (17).

The growth of breast cancer cells is controlled not only by ER and PR but also by plasma membrane-associated growth factor receptors. Two particularly important members of this large family are the epidermal growth factor receptor (EGFR), which is activated by the epidermal growth factor (EGF), and the human epidermal growth factor receptor-2 (HER2), both present in MCF-7 cells (7). Nevertheless, MCF-7 are considered to be moderate EGFR-expressing cell lines (2).

Table I. *Discoveries in breast cancer pathology through experiments using MCF-7 cells.*

Study by (Reference)	Discovery
Brooks *et al*. (15)	Significant amounts of 17β-estradiol-binding protein are present in a stable cell line derived from a human breast tumor (MCF-7).
Zhang *et al*. (28)	The growth advantage arises from increased tumor vascularization induced by VEGF121.
Aonuma *et al*. (30)	Tumor progression closely depends on angiogenesis and VEGF significantly contributes to malignant progression of the MCF-7 tumor cells through its potent angiogenic activity, independent on the bFGF productivity of tumor cells.
Dupont *et al*. (22)	Estrogen potentiates the effect of IGF-1 on IGF-IR signaling and its effects on certain cell cycle components.
Karpanen *et al*. (32)	VEGF-C facilitates tumor metastasis via the lymphatic vessels and tumor spread can be inhibited by blocking the interaction between VEGF-C and its receptor.
Buteau-Iozano *et al*. (14)	*VEGF* is a target gene for ER alpha and ER beta in breast cancer cells.
Hamelers *et al*. (16)	Estrogen responsiveness of MCF-7 cells is dependent on the secretion of an autocrine factor activating the IGF-IR.
Guo *et al*. (26)	VEGF stimulation of tumor angiogenesis and growth is mediated by both autocrine and paracrine mechanisms.
Lee *et al*. (29)	Reduced *VEGF* mRNA expression may be related to the antagonistic effect of tamoxifen on ER-positive breast cancer.
Akahane *et al*. (33)	VEGF-D functions both as an autocrine growth factor and a stimulator of angiogenesis in breast cancer.
Garvin *et al*. (27)	Tamoxifen and estradiol exert dual effects on the angiogenic environment in breast cancer by regulating cancer cell-secreted angiogenic ligands, such as VEGF and sVEGFR-1 and by affecting VEGFR2 expression of endothelial cells.
Barabutis *et al*. (12)	GHRH operates as a growth factor in breast cancer and probably other cancers as well.
Lee *et al*. (25)	There is evidence of a unique survival system in breast cancer cells by which VEGF can act as an internal autocrine (intracrine) survival factor through its binding to VEGFR1.
Ditttmer *et al*. (21)	hMSCs interfere with cell-cell adhesion and enhance migration of breast cancer cells by activating ADAM10 (a disintegrin and metalloprotease 10).
do Amaral *et al*. (23)	Changes in the extracellular matrix associated with long periods of time in 3D cell culture lead to the formation of a lumen in MCF-7 cell spheroids. The features of differentiation, such as lumen and budding formation, occur after long periods in 3D culture, even in the absence of exogenous extracellular compounds.
Klopp *et al*. (37)	MSCs increase the efficiency of primary mammosphere formation in malignant breast cells and decrease E-cadherin expression.
Comşa *et al*. (40)	Both tumor cells and VEGF alter the migration behavior of MSCs in a transmigration model indicating a role of tumor cell-derived VEGF to modulate the recruitment of MSCs into sites of angiogenesis.
Comşa *et al*. (11)	Both breast cancer cells and VEGF stimulate MSCs to form capillary-like structures indicating a role of tumor-derived VEGF in modulating their recruitment into sites of pathological vasculogenesis.
Martin *et al*. (17)	New insights into mechanisms governing IGF-1 signaling in breast cancer have been revealed.
Zhao *et al*. (39)	Human adipose-derived stem cells enhance the invasive activity of MCF-7 cells.
Gest *et al*. (9)	The Rac3/ERK-2/NF-κB signaling pathway is not functional because of the low expression of NF-κB subunits in MCF-7 cells.
D'Anselmi *et al*. (19)	MCF-7 cells cultured in egg white develop acini and mammary duct-like structures, after a transient growth effect.
Lian *et al*. (41)	Combinations of antiplatelet drugs may represent a promising strategy to prevent cancer metastasis.
Zhang *et al*. (38)	MSCs were confirmed to exist in human breast cancer tissues; breast cancer-MSCs may promote the proliferation and migration of breast cancer cells.

MCF-7 sub-lines demonstrate a wide divergence in the relative expression of ER, PR and HER2. The proportion of the dominant phenotype may be maintained by the growth conditions; the predominance of the ER-positive phenotype could be maintained by the presence of small amounts of estrogen in the fetal bovine serum. However, extended growth in the absence of estrogen would select for variants that rely on EGFR, HER2 and other stimulators of the signaling pathways (7). Interestingly, the ER-, PR- and HER2-negative (triple-negative) sub-lines seem to have the origin in the ER-positive MCF-7 cell line, too (18). This might form a useful model for understanding the triple-negative breast cancers from clinical practice. Thus, the generation of variants of a single cancer cell line might be able to recapitulate the development of multiple phenotypes in clinical cancer (7).

MCF-7 cells exhibit features of differentiated mammary epithelium: they are positive for epithelial markers, such as E-cadherin, β-catenin and cytokeratin 18 (CK18), and negative for mesenchymal markers, such as vimentin and smooth muscle actin (SMA) (19). MCF-7 parental cells also maintain the expression of other specific molecular markers of natural epithelial layers, such as claudins and zona occuldens protein 1 (ZO-1), among other proteins that constitute the intercellular junctions (20). On the other hand, MCF-7 cells are CD44-deficient (21) (Figure 1).

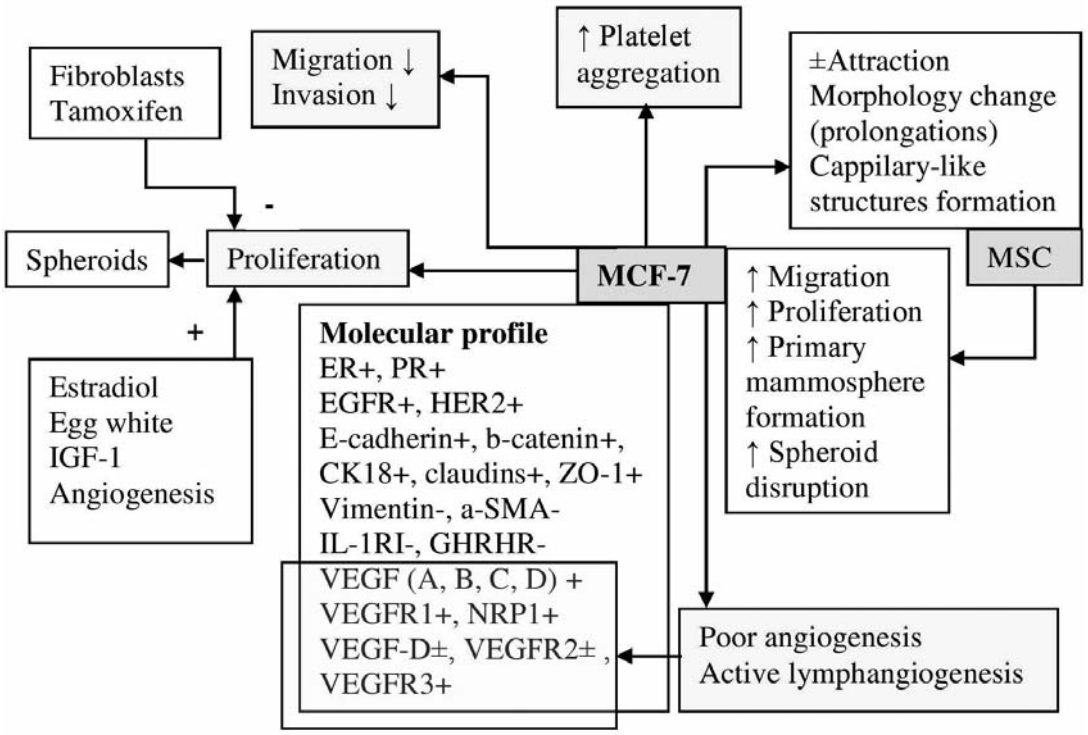

Figure 1. The molecular profile and properties of MCF-7, as well as the interaction between MCF-7 and the mesenchymal stem cells (MSC, mesenchymal stem cells; ER, estrogen receptor; PR, progesterone receptor; EGFR, epithelial growth factor receptor; HER2, human epithelial growth factor receptor 2; CK 18, cytokeratin 18; ZO-1, zona ocludens protein 1; a-SMA, alpha smooth muscle actin; IL-1RI, interleukin 1 receptor I; GHRHR, growth hormone releasing hormone receptor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; NRP1, neuropilin 1; IGF-1, insulin-like growth factor 1).

MCF-7pl (parental MCF-7 cells) and MCF-7A3 (cells selected for high sensitivity to interleukin (IL)-1β stimulus, uniform expression of C-X-C chemokine receptor type 4 (CXCR4) and stability of the interleukin 1 receptor, type I (IL1RI)) have similar basal expression of IL-1RI on the cell surface under non-stimulated conditions. However, when stimulated with IL-1β for 48 h, MCF-7pl cells loose the receptor from their surface, compared to MCF-7A3 cells. These results suggest that IL-1β may up-regulate the expression of the receptor or induce its faster recycling in MCF-7A3 cells (20).

MCF-7 cells do not express any growth hormone-releasing hormone receptor (GHRHR) and, thus, represent a useful system for assessing the effects of stimulating the expression of these receptors (12).

Interestingly, the cell growth control gene RAC3 has a minimal effect in MCF-7 cells, despite activating extracellular signal-regulated kinases (ERK). This can be explained by the fact that the signaling pathway cannot be fully effective because of the inactive state of the DNA transcription control of nuclear factor-κB (NF-κB) proteins in these cells (9).

Proliferation

Fibroblasts from normal breast tissue, but not conditioned medium from normal breast tissue, are able to inhibit the growth of MCF-7 cells suggesting complex paracrine interactions between the two cell types (8).

Estradiol has a significant effect to promote the growth of MCF-7 breast cancer cells. MCF-7 cells, however, are arrested for at least five days before being able to start a significant proliferation after the exposure to tamoxifen. This block is effective enough to prevent the stimulating effect of estradiol when cells are exposed to both agents simultaneously (8).

Apoptosis appears unlikely during serum deprivation as no evidence of cell death is noted after 4 days of culture in the absence of serum, despite the dramatic reduction in the baseline levels of cell proliferation (12).

MCF-7 cells cultured in egg white (EW) develop acini and mammary duct-like structures after a transient growth effect. It is noteworthy that a *de novo* synthesis and a concomitant release of high levels of β-casein occur in MCF-7 cells cultured in EW. Confocal microscopy shows that β-casein staining is localized into the lumen of the duct, while

transmission electron microscopy (TEM) demonstrates that its secretion is correctly oriented through images showing polarized MCF-7 cells with secretory vesicles placed in the apical part of the cell and directed toward the lumen (19).

After 48 h of stimulation, IGF-1 causes an approximately 1.7-fold increase in MCF-7 cellular proliferation, whereas E2 induces only an approximately 1.3-fold increase. In contrast, the combination of IGF-1 and E2 induce a four- to five-fold increase suggesting that E2 potentiates the effect of IGF-1 on cellular proliferation (22).

MCF-7pl cells proliferate and form compact colonies with typical epithelial polygonal shape in close contact with each other. Few cells, at the border of the colonies, show fibroblastic shape and move away from the colony mass. In contrast, colonies formed by MCF-7A3 cells are not compact, they do not show tight contacts with neighboring cells and many of them move away from the parental colony, alone or in groups. When MCF-7pl cells are stimulated with IL-1β for 48 h, more fibroblastoid cells are seen scattered from the border of the colonies showing cytoplasmic projections suggestive for a migratory phenotype. These structural changes are clearly evident in the great majority of MCF-7A3 cells stimulated with IL-1β. In addition, in these cultures, numerous small satellite colonies -close to larger colonies- are observed. In few of the remaining colonies, cells are seen in the process of detaching from each other. In the absence of the cytokine, MCF-7A3 cells show a higher than 2-fold increase of the number of colonies formed in respect to those formed by MCF-7pl cells. After stimulation with IL-1β for 48 h, MCF-7A3 cells show a further increase to 3-fold compared to the parental cells due to the increased number of satellite colonies formed in these cultures (20).

Spheroid Formation

MCF-7 cells show many features of normal breast epithelial cells, including the ability to generate multicellular 3D-aggregates that can mature to lumen-containing spheroids. The spheroids of the MCF-7 lineage are described as a mass of cells with disorganized nuclei, strong cell-to-cell bonds and absence of lumen formation. Different cell death processes occur mainly in the medullary region. As many cell death processes other than necrosis were observed, it is suggested that the 'cell death region' is a more appropriate term than the 'necrotic nucleus'. However, the gradual cell clearance in the cell death region in these spheroids culminates in a polarized cortical monolayer (23). E-cadherin is the major protein that mediates MCF-7 cell–cell adhesion in spheroids (21).

Migration/Invasion

The parental MCF-7 cells do not usually migrate or invade (9). An autocrine loop exists for the vascular endothelial growth factor (VEGF) to induce breast cancer cell migration/invasion. MCF-7 cells express lower levels of VEGF than MDA-MB-231 cells, which have high invasive and migration capacities. Without estrogen supplementation, MCF-7 cells do not induce metastasis in mice and have a low capacity of migration *in vitro* (13).

Interestingly, more than 90% of the subpopulation MCF-7A3 respond to the IL-1β stimulation with uniform, programmed changes of cell shape, scattering, proliferation, chemokinesis and invasiveness, concomitantly with sequential delocalization of E-cadherin from the cell membrane, its accumulation in the cytoplasm and its degradation, suggesting that this sub-population acquires a migratory/invasive phenotype (20).

MCF-7 and Angiogenesis/Lymphangiogenesis

The MCF-7 human breast cancer cell line was found to express mRNA, although at different levels, for all four *VEGFs* (A, B, C, D), as detected by RT–PCR. They secrete immunodetectable but low levels of VEGF-A and VEGF-C and very low levels of VEGF-D (24).

On the other hand, VEGF receptor 1 (VEGFR1) and neuropilin-1 (NRP1) are abundantly expressed in the MCF-7 cells, both *in vitro* and *in vivo* (25, 26), while VEGFR2 is poorly expressed (25). Some authors conclude that VEGFR2 was not detected in MCF-7 cells neither in the absence nor in the presence of hormones (27), while others note that VEGFR2 was only detected in tumor endothelial cells, but not in the carcinoma cells of the MCF-7 tumors (26).

The growth of MCF-7 tumors appears to be limited by angiogenesis (28). The reduction in VEGF or VEGFR1 expression induces significant cell death in the MCF-7 cells, whereas the reduction in NRP1 or VEGFR2 expression has no significant effects on the survival of these cells (25). VEGFR1 is expressed internally in MCF-7 cells (25) suggesting that MCF-7 cells express only secreted (s)VEGFR1 and virtually no cell membrane-bound VEGFR1 (27).

The VEGF induction in the MCF-7 cells is mediated by activation of ERα (14) and that is why tamoxifen was observed to reduce VEGF mRNA expression in MCF-7 cells. This partly explains the antagonistic effect of tamoxifen on ER-positive breast cancers (29). At any rate, the MCF-7 cells prove a poor angiogenic potential, which could cause, at least partially, the lack of tumorigenicity of this cell line (30).

MCF-7 cells generate estrogen-dependent solid tumors and produce metastases to local and distant lymph nodes (31). VEGF-C over-expression in MCF-7 mammary tumors strongly and specifically induces the growth of tumor-associated lymphatic vessels but does not have major effects on tumor angiogenesis. Unlike lymphatic endothelial cells in normal adult tissues, the lymphatic endothelial cells associated with the MCF-7 tumors are actively proliferating.

On the basis of this information, it is speculated that most of the peri- and intra-tumoral lymphatic vessels are generated through the proliferation of the endothelial cells of pre-existing lymphatic vessels (32).

The MCF-7 cells seem to express VEGF-D, VEGFR2 and VEGFR3 by RT-PCR but the VEGF-D and VEGFR2 proteins cannot be detected by western blot analysis on MCF-7 cell lysates (33). VEGF-D mRNA is up-regulated in MCF-7 cells incubated in medium containing 17β-estradiol (34). Heregulin beta-1 potently induces the up-regulation of *VEGF-C* mRNA and VEGF-C protein in the MCF-7 cells through a novel signaling pathway from HER2 receptor, p38 mitogen-activated protein kinases (MAPK), to the subsequent activation of NF-κB cascade, resulting in DNA transcription (35).

It has been demonstrated that VEGF-C has the ability to turn poorly invasive human MCF-7 breast cancer cells into a metastatic cell line in a nude mouse model for human breast cancer. In addition, the results confirm the association of VEGF-C expression with increased tumor lymphangiogenesis. It seems that over-expression of VEGF-C does not affect the proliferation or estrogen responsiveness of MCF-7 cells in terms of growth rate *in vitro*. It does, however, increase tumor size and rate of tumor formation *in vivo* (36).

Interactions with the Human Mesenchymal Stem Cells (hMSCs)

MCF-7 cells are able to attract hMSCs by secreting a chemoattractant. On the other hand, hMSCs may stimulate MCF-7 cells to migrate faster by secreting a protein that stimulates MCF-7 migration (Figure 1). Whether the nuclear E-cadherin proteins play a role in the effect of hMSCs on MCF-7 migration is not clear yet (21). Areas of lower E-cadherin expression were identified in tumors containing MSCs but they were absent in tumors without co-injected MSC (37). hMSCs enhance the migratory activity of MCF-7 cells by approximately two-fold. In the presence of conditioned medium (CM) from hMSCs, the migratory activity of MCF-7 cells is also increased (21). It is hypothesized that 10 to 20% CM may significantly promote MCF-7 cancer cell migration (38). Some authors suggest that MCF-7 tumor growth is not significantly increased by co-injection of MSC; however, the MCF-7 tumors are detected earlier when tumor cells are co-injected with MSCs (37). Others prove that breast cancer MSs-conditioned medium (BC-MSC-CM) significantly stimulates cancer cell proliferation indicating that BC-MSC-CM may have certain increasing effects on the growth of breast cancer *in vitro* (38). In addition, human adipose-derived stem cells (hADSCs) show significantly higher chemotaxis and invasive effects on MCF-7 cells than the cells treated with adipogenic induction (39).

MSCs increase the capacity of MCF-7 to form primary mammospheres. Both direct exposure to MSCs, as well as MSC-conditioned media, promote mammosphere formation from MCF-7 demonstrating that MSC secrete a sphere-promoting factor to which these cells are sensitive (37). MSCs integrate into breast cancer mammospheres and decrease E-cadherin expression in the estrogen receptor-positive luminal E-cadherin-positive cells (MCF-7) (37) and, probably, that is why the MCF-7 spheroids lose their normal morphology and appear disrupted after addition of hMSCs (21).

CM-MCF-7 in the presence of MCF-7, seems to attract significantly less MSCs than the MCF-7 medium (low glucose DMEM containing 10% FBS, 2 mM glutamine, 0.01 mg/ml insulin and 1% penicillin/streptomycin mix) alone. No difference in attracting MSCs either between CM alone and MCF-7 medium or CM alone and CM in the additional presence of MCF-7 cells was noted. MCF-7 or CM have a similar effect on the MSCs' morphology to VEGF determining the appearance of longer and thinner prolongations comparing to those of the MSCs in contact with plasma. It seems that both MCF-7 and VEGF alter the migration behavior of MSCs indicating a role of tumor cell-derived VEGF to modulate the recruitment of MSCs into sites of angiogenesis (40).

MSCs have a clear tendency to organize into clusters and to form capillary-like structures in Matrigel both in the presence of VEGF and the MCF-7 cell suspension, thus indicating a role for the tumor-derived VEGF in this process. By contrary, MSCs do not organize in clear capillary-like structures in the presence of MCF-7 CM. MSCs develop long and thin prolongations interconnecting each other following exposure to VEGF. A similar pattern can be noted when MSCs are co-cultured with MCF-7 cells. The evaluation of MSCs in immunofluorescence after 48 h of incubation with VEGF, CM and the MCF-7 cell suspension shows that, independent of the conditions of incubation, MSCs do not express CD31. As a conclusion, both MCF-7 cells and VEGF stimulate MSCs to form capillary-like structures proving that MCF-7-derived VEGF is involved in regulating the tumor vasculogenesis in breast cancer (11). Interestingly, most of the MCF-7 cells co-cultured with MSCs are identified as adherent single cells, without organizing into clusters (11, 40), suggesting that MSCs might lower the level of adhesion molecules in MCF-7.

Platelet Aggregation

MCF-7 cells induce platelet aggregation in a similar manner to collagen, a classic inducer of platelet aggregation. MCF-7 cells stimulate this process in a concentration-dependent manner, up to a maximal concentration of 5×10^6 cells/ml. The pathways, including the glycoprotein receptor complexes GPIb-IX and GPIIb/IIIa, are activated during

MCF-7 tumor cell-induced platelet aggregation and the inhibition of the GPIb-IX and GPIIb/IIIa pathways represses MCF-7 action on platelets. MCF-7-induced platelet aggregation leads to activation of the ADP pathway and the process is inhibited by the ADP scavenger, apyrase. It has been observed that a combination of GPIb-IX, GPIIb/IIIa and ADP pathway inhibitors exhibits a significant repression of the MCF-7 tumor cell-induced platelet aggregation when compared with inhibition of a single pathway alone (41).

Future Directions

There is considerable ethical pressure on scientists to reduce or eliminate the use of animals in laboratory research and primary cell culture may be one way forward, especially in preclinical drug testing (3).

MCF-7 cells have the high advantage that they are very well characterized, due to the impressive number of papers that have described them. This strong experience with MCF-7 allows researchers to use this cell line for bringing more light into breast cancer pathogenesis and treatment protocols through reliable *in vitro* assays.

MSCs may provide a new approach for cancer therapy. Because of the existing controversies regarding the relation between MSCs and tumors and in order to explain the involvement of MSCs in tumorigenesis and cancer progression (38), studies are required before MSCs may be widely used in clinical cancer therapy. The model of cooperation between MSCs and MCF-7 cells could solve this problem by offering valuable information needed to clarify many aspects in this area of research.

Because of their ease of use, there is no doubt that established cell lines will continue to be used as models for breast cancer. However, it is essential that researchers understand their limitations and take them into consideration when designing experiments and interpreting results (3).

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