Обзорные статьи

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STEM CELL THERAPY FOR HEREDITARY BREAST CANCER

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Both hereditary and sporadic breast cancers may develop through dysregulation of self-renewal pathways of normal mammary stem cells. Networks of proto-oncogenes and tumor suppressors that control cancer cell proliferation also regulate stem cell self-renewal and possibly stem cell aging. Breast cancer susceptibility gene (BRCA1) is a nuclear phosphoprotein expressed in many nuclear processes, including stem cell regulator, DNA damage repair, recombination, transcription, ubiquitination, cell cycle checkpoint enforcement, and centrosome regulation. In this study, we report on recent advances on the functions of embryonic, fetal, and adult stem cell progenitors for hereditary breast cancer therapies. Several molecular targeting therapies are described by activation and blocking distinct developmental signaling cascade elements, such as BRCA1, EGFR, hedgehog, Wnt/β-catenin, and/or Notch pathways, which are frequently upregulated in cancer progenitor cells during the initiation and development of breast cancer.

Introduction

Breast cancer results from the dysregulation of self-renewal pathways of normal mammary stem cells. Both hereditary and sporadic breast cancers may develop through deregulation of stem-cell self-renewal pathways [1]. The hereditary breast cancer (HBC) includes genetic alterations of various susceptibility genes such as *TP53, ATM, PTEN or MSH2, MLH1, PMS1, PMS2, CDH1, MSH3* and *MSH6, BRCA1* and *BRCA2* [2]. Germline mutations in *BRCA1* and *BRCA2* account for the majority of families with multiple cases of breast and/or ovarian cancer and also at least 10 % of cases below the age of 40 years in Ukraine [3]. Most *BRCA1* carcinomas have the basal-like phenotype and are high-grade, highly proliferating, estrogen receptor-negative and HER2-negative breast carcinomas, characterized by the expression of basal markers such as basal keratins, P-cadherin and epidermal growth factor receptor [4, 5]. The *BRCA1* carcinomas frequently carry p53 mutations. One of the key functions of *BRCA1* is to act as a stem cell regulator [6]. It regulates the development of the estrogen-receptor-negative stem cells into estrogen-receptor-positive cells. When *BRCA1* is missing, genetically unstable stem cells accumulate and then may develop into breast cancers. In this review, we report on recent advances on the functions of adult stem cells for hereditary breast cancer therapies.
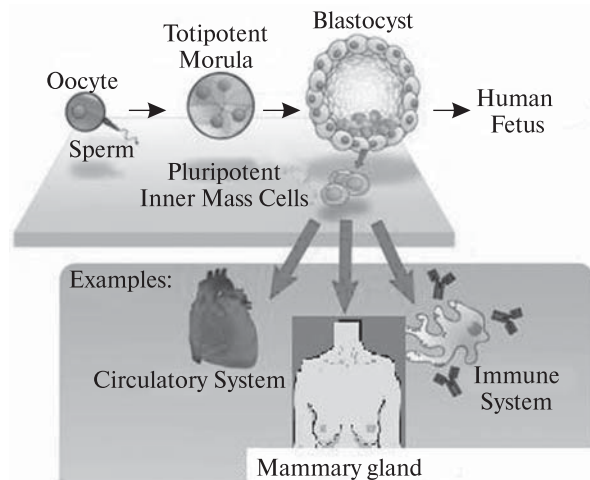
Stem cells types in mammary gland

Stem cells are undifferentiated cells for which the mitotic progeny have the potential to generate differentiated cells throughout the lifespan. Self-renewal and differentiation potential is the feature of stem cells. However, distinct stem cell types have been established from embryos and identified in the fetal tissues and umbilical cord blood (UCB), as well as in many adult mammalian tissues and organs, such as bone marrow (BM), breast, brain, skin, eyes, heart, kidneys, lungs, gastrointestinal tract, pancreas, liver, ovaries, prostate, and testis [7].

Embryonic stem cell lines (ES cell lines) are cultures of cells derived from the epiblast tissue of the inner cell mass (ICM) of a blastocyst or earlier morula stage embryos (Figure). A blastocyst is an early stage embryo-approximately four to five days old in humans and consisting of 50−150 cells. ES cells are pluripotent and give rise during development to all derivatives of the three primary germ

layers: ectoderm, endoderm and mesoderm. A human embryonic stem cell is also defined by the presence of several transcription factors and cell surface proteins. The transcription factors Oct-4, Nanog, and SOX2 form the core regulatory network that ensures the suppression of genes that lead to differentiation and the maintenance of pluripotency [8]. The cell surface antigens most commonly used to identify hES cells are the glycolipids SSEA3 and SSEA4 and the keratan sulfate antigens Tra-1−60 and Tra-1−81. Adult stem cells are undifferentiated cells found throughout the body after embryonic development that divide to replenish dying cells and regenerate damaged tissues. Most adult stem cells are lineage-restricted (multipotent) and are generally referred to by their tissue origin (mesenchymal stem cell, adiposederived stem cell, endothelial stem cell, etc.) (Figure). The pluripotent adult stem cells are rare and generally small in number but can be found in a number of tissues including mammary glands [6−8]. A great deal of adult stem cell research has focused on clarifying their capacity to divide or self-renew indefinitely and their differentiation potential. The use of adult stem cells in research and therapy is not as controversial as embryonic stem cells, because the production of adult stem cells does not require the destruction of an embryo. Additionally, because in some instances adult stem cells can be obtained from the intended recipient, (an autograft) the risk of rejection is essentially non-existent in these situations.

Cancer stem cells originate from the transformation of normal epithelial stem cells in mammary gland. Cancer stem cells may provide targets for the development of cancer prevention strategies. Furthermore, because breast cancer stem cells may be highly resistant to radiation and chemotherapy, the development of more effective therapies for this disease may require the effective targeting of this cell population.

Mammary gland is a dynamic organ that undergoes significant developmental changes during pregnancy, lactation, and involution. In the neonate, Mammary gland development is not complete, consisting only of a limited network of ducts. More specifically, the postnatal growth of the mammary gland during puberty, pregnancy, and lactation might notably be induced by estrogenic hormones that may regulate epithelial stem cell behavior in paracrine

Pluripotent, embryonic stem cells originate as inner mass cells within a blastocyst

fashion. In this matter, the human adult mammary gland adopts a lubulo-alveolar structure that is composed of different epithelial cell types, including alveolar epithelial cells, contractile myoepithelial cells forming the basal layer of ducts and alveoli, and specialized epithelial cells constituting the luminal layer of ducts. Distinct undifferentiated and multipotent stem cell subpopulations have been identified in the mammalian mammary epithelium within the niches localized near the basement membrane [8]. These stem cell subtypes, which can express either estrogen receptor-α (ER-α-positive cells) or undetectable ER-α levels (ER-α-negative cells), as well as specific stem cell markers, including Sca-1, K19, and Msi-1, are able to give rise to myoepithelial and luminal epithelial cells in vitro [1]. Moreover, it has also been shown that the propagation of human undifferentiated mammary epithelial cells derived from the reduction mammoplasties under the form of nonadherent mammospheres in vitro, results in the generation of three mammary epithelial cell types [6].

These undifferentiated mammary epithelial cells may generate a functional ductal-alveolar structure resembling the mammary tree in reconstituted three-dimensional Matrigel culture system [7]. In addition, it has been observed that a suprabasal-derived mammary epithelial cell line, which was able to self-renew and differentiate into myoepithelial and luminal epithelial cells, could also form terminal duct lobular unit-like structures within a reconstituted basement membrane [8].

Role of estrogen and BRCA1 gene in hereditary breast cancer

Breast cancer susceptibility gene (*BRCA1*) is a nuclear phosphoprotein expressed in many nuclear processes, including DNA damage repair, recombination, transcription, ubiquitination, cell cycle checkpoint enforcement, and centrosome regulation [9]. The *BRCA1* is mutated in about one half of all hereditary breast cancer cases, and its expression is frequently decreased in sporadic cancers [2]. Women with hereditary breast and ovarian cancer due to *BRCA1* mutations are born with a mutation in one *BRCA1* allele, but only develop cancer after mutation or allelic loss of the other *BRCA1* allele. Thus, this is in contrast to inherited germline diseases, in which the genetic defect is present in all cells. Breast cancers arising in carriers of germline *BRCA1* mutations frequently have a basal-like phenotype [4, 5, 9]. Basal-like cancers are characterized by high histological grade, central necrotic areas, foci with metaplastic differentiation, lack of ER and PR and HER2 (ErbB2) expression, and consistent positivity for basal markers, including CK5/6, CK14, and EGFR.

Estrogen exposure is considered a significant risk factor for breast cancer development. Estrogen receptor (ER) alpha is expressed at low levels in normal epithelia, and its expression is dramatically up-regulated as transformation progresses during mammary hyperplasia and adenocarcinoma development [3]. About 70 % of breast cancers express ER and are estrogen-dependent for growth. The *BRCA1* inhibits signaling by the ligand-activated ER through the estrogen-responsive enhancer element and block the transcriptional activation function AF-2 of ER-alpha. In other hand, *BRCA1* suppresses estrogen-dependent transcriptional pathways related to mammary epithelial cell proliferation and that loss of this ability contributes to tumorigenesis. This gene regulates Akt signaling and the PI3K/Akt pathway modulates the ability of *BRCA1* to repress ER-alpha, in part through serine phosphorylation events in the activation function-1 domain of ER-alpha [1, 3, 10].

Regulation of stem cells

Networks of proto-oncogenes and tumor suppressors that control cancer cell proliferation also regulate stem cell self-renewal and possibly stem cell aging [3]. Proto-oncogenes promote regenerative capacity by promoting stem cell function but must be balanced with tumor suppressor activity to avoid neoplastic proliferation. Conversely, tumor suppressors inhibit regenerative capacity by promoting cell death or senescence in stem cells. The regulation of the self-renewal, differentiation, and migration of mammary stem cells and their progenitors that are localized in the mammary glands appears to be assumed through distinct developmental signaling pathways such as hormones, EGF, hedgehog, Wnt/β-catenin, Notch, and Bmi-1 [1]. The hedgehog signaling components PTCH1, Gli1, and Gli2 are highly expressed in normal human mammary stem/progenitor cells cultured as mammospheres and that these genes are downregulated when cells are induced to differentiate. Activation of hedgehog signaling increases mammosphere-initiating cell number and mammosphere size, whereas inhibition of the pathway results in a reduction of these effects [6]. These effects are mediated by the polycomb gene Bmi-1. Furthermore, there is strong evidence suggesting that aberrant activation of Wnt signaling induces mammary tumors from stem/progenitor cells, and that Wnt exerts its oncogenic effects through LRP5/6-mediated activation of beta-catenin and mTOR pathways. Inactivation of Notch signaling may contribute to mammary carcinogenesis by deregulating the self-renewal of normal mammary stem cells. Polycomb family proto-oncogene, Bmi-1, is consistently required for the self-renewal of diverse adult stem cells, as well as for the proliferation of cancer cells in the same tissues. The Bmi-1 promotes stem cell self-renewal partly by repressing the expression of *Ink4a* and *Arf*, tumor suppressor genes that are commonly deleted in cancer. Despite ongoing Bmi-1 expression, *Ink4a* expression increases with age, potentially reducing stem cell frequency and function [7, 8]. Increased tumor suppressor activity during aging therefore may partly account for age-related declines in stem cell function. The studies demonstrated that *BRCA1* plays a critical role in the differentiation of ER-negative stem/progenitor cells to ER-positive luminal cells. Defect of *BRCA1* gene may result in the accumulation of genetically unstable breast stem cells, providing prime targets for further carcinogenic events, because *BRCA1* also plays a role in DNA repair. Knockdown of *BRCA1* in primary breast

epithelial cells leads to an increase in cells displaying the stem/progenitor cell marker ALDH1 and a decrease in cells expressing luminal epithelial markers and estrogen receptor [1, 6–8]. Thus, networks of proto-oncogenes and tumor suppressors have evolved to coordinately regulate stem cell function throughout life. Imbalances within such networks cause cancer or premature declines in stem cell activity that resemble accelerated aging and breast cancer.

Production of patient-specific pluripotent stem cells

The generation of patient-specific pluripotent stem cells has the potential to accelerate the implementation of stem cells for clinical treatment of breast cancer. Induced pluripotent stem (iPS) cells have recently been established by transfecting mouse and human fibroblasts with the transcription factors Oct3/4 (Pouf51), Sox2, Klf4 and c-Myc, known to be expressed at high levels in embryonic stem (ES) cells [11]. Transfection is typically achieved through viral vectors, such as retroviruses. After 3–4 weeks, small numbers of transfected cells begin to become morphologically and biochemically similar to pluripotent stem cells, and are typically isolated through morphological selection, doubling time, or through a reporter gene and antibiotic selection. The iPS cells are believed to be identical to natural pluripotent stem cells, such as embryonic stem cells in many respects, such as the expression of certain stem cell genes and proteins, chromatin methylation patterns, doubling time, embryoid body formation, teratoma formation, viable chimera formation, and potency and differentiability, but the full extent of their relation to natural pluripotent stem cells is still being assessed. These cells were first produced in 2006 from mouse cells and in 2007 from human cells [12, 13]. Four key pluripotency genes essential for the production of pluripotent stem cells were isolated; Oct-3/4, SOX2, c-Myc, and Klf4. Cells were isolated by antibiotic selection for Fbx15+ cells. However, this iPS line showed DNA methylation errors compared to original patterns in ESC lines and failed to produce viable chimeras if injected into developing embryos. Using Nanog which is an important gene in ESCs, DNA methylation patterns and producing viable chimeras (and thereby contributing to subsequent germ-line pro-

duction) indicated that Nanog is a major determinant of cellular pluripotency. Unfortunately, one of the four genes used (namely, c-Myc) is oncogenic, and 20 % of the chimeric mice developed cancer. In a later study, Yamanaka reported that one can create iPSCs even without c-Myc. The process takes longer and is not as efficient, but the resulting chimeras didn't develop cancer [11]. With the same principle used earlier in mouse models, Yamanaka had successfully transformed human fibroblasts into human pluripotent stem cells using the same four pivotal genes: Oct3/4, Sox2, Klf4, and c-Myc with a retroviral system. Thomson and colleagues used OCT4, SOX2, NANOG, and a different gene LIN28 using a lentiviral system. The viral transfection systems used insert the genes at random locations in the host's genome; this is a concern for potential therapeutic applications of these iPSCs, because the created cells might be susceptible to cancer. Members of both teams consider it therefore necessary to develop new delivery methods [12].

The generation of iPS cells is crucial on the genes used for the induction. The Oct-3/4 and certain members of the Sox gene family (Sox1, Sox2, Sox3, and Sox15) have been identified as crucial transcriptional regulators involved in the induction process whose absence makes induction impossible. Additional genes, however, including certain members of the Klf family (Klf1, Klf2, Klf4, and Klf5), the Myc family (C-myc, L-myc, and N-myc), Nanog, and LIN28, have been identified to increase the induction efficiency [13, 14].

Stem cell-based therapy

Embryonic, fetal, amniotic, umbilical cord blood and adult stem cells could be used for treating numerous genetic and degenerative disorders. Among them, age-related functional defects, cancers, Parkinson's and Alzheimer's diseases, hematopoietic and immune system disorders, heart failures, chronic liver injuries, diabetes, arthritis, and muscular, skin, lung, eye, and digestive disorders as well as aggressive and recurrent cancers could be successfully treated by stem cell-based therapies. Clinical transplantation procedures for stem cells, which depend on patient state and diagnosis, generally involve the i.v. injection or subcutaneous administration of a specific number of stem cells directly into therapeutically targeted areas. In addition, the

high plasticity and migratory potential of BM stem cells also offer the possibility of mobilizing them in vivo or injecting these stem cell types in circulation to regenerate the particular functional progenitors for the tissue regeneration [15]. Moreover, gene-based strategies involving modifications or replacement of a particular gene product, such as MDR1, in stem cells and their more differentiated progenitors before their transplantation might now be conceived for the treatment of cancer [16]. Nuclear transfer, in which the nucleus from donor somatic cells is transferred into an enucleated oocyte to obtain the pluripotent embryonic stem cells, offers another alternative source for the derivation of primitive stem cells for cell replacement therapy when no donor organ is available for transplantation [13]. However, molecular targeting of tumorigenic cascade elements in tissue-specific cancer progenitor cells, which are derived from the malignant transformation of adult stem cells, also represents a novel approach for the treatment of diverse metastatic and incurable cancer types by combination therapies [14].

The cancer stem cells play a critical role in both initiation and relapse of the cancers as they are resistant to the most of cytotoxic agents and able to proliferate indefinitely. Several resistance mechanisms have been proposed, including amplified checkpoint activation and DNA damage repair as well as increased Wnt/beta-catenin and Notch signaling [7, 8]. Moreover, several studies have been carried out with a variety of cancer cell line types and on different animal models to identify new therapeutic targets to block the growth and/or survival of the cancer cells. Among them, the molecular targeting of distinct oncogenic signaling elements, which are activated in the cancer cells during the progression of numerous cancer, represents a promising strategy for the development of new chemopreventive treatments and combination therapies against some aggressive and metastatic cancers. Inactivation and/or activition of diverse hormones, growth factors, cytokines and chemokines (androgens, estrogens, EGF and TGF-α/EGFR, IGF/IGFR, SHH/SMO, Wnt/β-catenin, Notch, TGF-β, and SDF-1/CXCR4), and tumorigenic signaling elements (telomerase, phosphatidylinositol 3-kinase [PI3K]/Akt, NF-κB, and Myc-1) may contribute to the sustained growth and survival of stem cells, as well as their malignant transforma-

tion during the initiation and cancer progression [15, 16]. Therefore, their molecular targeting is of importance to the elimination of cancer progenitor cells, thereby inducing a complete tumor regression and cancer remission. There is described a brief description of new therapeutic drugs that are able to block the specific growth factor signaling cascades that are frequently deregulated in the stem cell-derived cancer progenitor cells, as well as the advantages that are associated with the use of high-dose chemotherapy (HDCT) with hematopoietic cell support.

Growth Factor Signaling Inhibitors

EGFR Family Member Inhibitors. Inactivation of EGFR (erbB2) might represent a potent strategy, alone or in combination with other conventional treatments for numerous aggressive cancer forms [17−19]. Among the selective agents targeting EGFR signaling, there are antibodies or antisense oligonucleotides directed against EGFR or its ligands EGF and TGF-, anti-ErbB2 antibody trastuzumab (Herceptin) and the selective EGFR tyrosine kinase inhibitors such as AG1478, gefitinib and erlotinib [17]. These agents may induce the inhibition of the growth, invasiveness, and apoptotic death of diverse cancer cell types by counteracting distinct mitotic cascades, including MAPK, PI3K/Akt, NF-κB, phospholipase Cγ, and Shc [19].

Hedgehog, Wnt/β-catenin, and Notch Signaling Inhibitors. The inactivation of hedgehog signaling by using either SMO signaling element inhibitor, cyclopamine alkaloid, or the anti-SHH antibody has been observed to result in vitro and in vivo in a growth inhibition and the apoptotic death of the metastatic cancer cells, whereas normal cells were insensitive to the cytotoxic effects of these agents [17]. In addition, molecular targeting of the canonical Wnt/β-catenin signaling elements constitutes another anticarcinogenic strategy for the treatment of breast cancer [20]. The Wnt protein inhibitors (such as Wnt-inhibitory factor-1), or repressors disrupting nuclear lymphocyte enhancer factor/T-cell factor/β-catenin complexes might counteract the intracellular and nuclear accumulation of β-catenin, thereby inhibiting the proliferation of cancer cells that is induced through the Wnt/β-catenin pathway. Similarly, the inhibition of the Notch signaling cascade, which

also appears to participate in developing certain cancer types, including acute T-cell lymphoblastic leukemia and lymphoma, medulloblastoma, and mucoepidermoid, colorectal, pancreatic, mammary, ovarian, and lung carcinomas, may also represent another targeting approach in the therapeutic interventions against these hyperproliferative disorders [21]. For instance, the inhibition of the β- and γ-secretases, whose proteases can cleave the intracellular domain of the Notch transmembrane receptor, thereby permitting its translocation into the nucleus, where it participates in the transcriptional activation of genes, may notably represent a potent therapeutic target for these malignant disorders [17, 18].

BRCA1 gene therapy. Although *BRCA1* is only mutated in a small percentage of breast or ovarian cancers, the majority of sporadic breast and ovarian cancers appear to express low levels of *BRCA1* messenger RNA and protein. This appears to be a consequence of loss of heterozygosity and promoter methylation of the remaining *BRCA1* allele. This finding is important because it indicates that restoration of normal «wild-type» *BRCA1* expression levels in many sporadic cancers may inhibit tumors by a «genetic correction» strategy, wherein the loss of *BRCA1* expression contributes to tumorigenesis. These results constitute the scientific basis for testing *BRCA1* gene therapy in patients with sporadic breast, ovarian, and prostate cancers that lack specific point mutations in the *BRCA1* gene [1−5].

Many approaches that use viral and nonviral delivery systems have been employed to introduce genes into tumor cells, thus changing their malignant phenotype. Several different *BRCA1* viral vectors have been constructed and tested for efficacy in preclinical xenograft models of breast and ovarian cancer. Initial studies of a *BRCA1* retroviral vector employed a complementary DNA that encoded a splice variant vector that eliminates the first 71 amino acids of the human protein, termed BRCA1sv [22]. Studies of both growth inhibition and DNA repair do not identify cellular or molecular differences between *BRCA1sv* and *BRCA1* complementary DNAs [23]. Intaperitoneal injection of either *BRCA1*sv or a full-length BRCA1 retroviral vector into ovarian cancer or breast cancer xenografts in nude mice produces tumor inhibition. These studies show that treatment of established SKOV3 or PA-1 ovarian cancer nude mice xenografts with either the full-length or the splice variant *BRCA1* retroviral vector results in tumor suppression. Necropsies showed that PA-1 tumor-bearing mice treated with control media or low-dose LXSN-*BRCA1sv* died with large intra-abdominal tumors and ascites, whereas mice with high-dose LXSN-*BRCA1sv* treatments died of lung metastasis with significantly smaller abdominal tumor [6−9, 23, 24]. The published phase 1 trial of *BRCA1sv* retroviral gene therapy demonstrated gene transfer and expression of the intraperitoneally injected LXSN-*BRCA1sv* vector. The vector was moderately stable in the peritoneum of these patients, and antibody formation was rare. The phase 2 trial performed in a group of patients with lower tumor burdens [22], however, demonstrated that tumor size and immune status strongly influence patient response to retroviral vectors, and that vectors packaged in mouse cells are not sufficiently stable to treat patients with small volume intraperitoneal ovarian cancer.

Combination Therapies. The simultaneous inhibition of diverse hormone and growth factor signaling pathways, including *BRCA1*, ER, AR, IGFR, EGFR, hedgehog, Wnt/β-catenin, Notch, and/or G-protein-coupled receptors, as well as VEGFR and PDGFR cascades, which can act in cooperation by stimulating the growth, invasion, and metastatic spread of cancer cells at distant sites during the different stages of cancer progression, may also constitute more effective therapies against the aggressive and highly metastatic cancer forms [15, 16]. As a matter of fact, some works have revealed that complex cross-talks may occur among the AR, ER-α/ER-β, EGFR/ErbB2 signaling cascades in breast cancers [17, 18]. The combination of the agents that are able to block these tumorigenic pathways may be more effective to treat these epithelial malignancies as a single antihormonal therapy. Simultaneous blockade of the EGFR and hedgehog pathways could represent a more effective and safe therapeutic treatment against certain metastatic cancer forms by decreasing the secondary effects that are associated with the use of high doses of these agents. Similarly, it has been reported that the activation of EGFR might lead to the cellular accumulation of β-catenin, and Notch and EGFR signaling may cooperate for the sustained growth and invasion of certain cancer cell types [19, 20].

Since the metastatic spread of diverse tumor cells, including those from glioblastomas, melanomas, and pancreas, breast, and prostate cancers to other specific tissues/organs, such as lymph nodes, bone, lungs, and/or liver, appears to be governed by the expression of diverse angiogenic factors, such as VEGF-VEGFR system, matrix metalloproteinases, urokinase-type plasminogen activator (uPA), cyclooxygenase-2 (COX-2), chemokines, and surface adhesion molecules, their molecular targeting may also constitute another adjuvant cancer therapy [15]. In this matter, the specific blockade of the SDF-1-CXCR4 axis by using a specific anti-SDF-1 antibody, anti-CXCR4 antibody, or CXCR4 antagonist (TC14012, TN14003, or AMD3100) has notably been observed to prevent the metastatic spread and interfere with the homing of breast and prostate cancer epithelial cells at their target metastatic sites, including lymph nodes, bone, and lungs [16]. The results from preclinical studies have also indicated that the use of the EGFR inhibitors in combination with COX-2 inhibitor or photodynamic treatment or as chemo- and radiosensitizers resulted in more effective chemopreventive and curative treatments for patients with advanced and metastatic cancer forms [17]. It is noteworthy that the sequence of treatment with the EGFR inhibitor and chemotherapy appears to be a critical factor that should be considered for clinical application. In contrast, the treatment of cells with the EGFR inhibitor before chemotherapy induced an antagonistic effect instead. In addition, since the resistance of several metastatic cancer cells to radiotherapy and chemotherapy has been associated with the aberrant response elements in ceramide and caspase cascades, targeting these apoptotic pathways also may represent another antitumoral strategy [18]. Altogether, these recent studies have indicated that molecular targeting of EGFR signaling, alone or in combination with other cytotoxic agents, may constitute a putative strategy for conceiving more effective clinical treatments against a variety of aggressive cancers [19].

High-Dose Cancer Therapy Plus HSCs. Stem cell transplantation may also constitute an option as adjuvant therapy for cancer, particularly in the patients receiving high doses of chemotherapeutic agents and/or radiation that, along with killing cancer cells, cause the severe damage to normal tissues and/or destroy the hematopoietic cells. Thus, the stem cell transplants might replace the endogenous stem cells destroyed by high-dose cancer treatment, thereby producing healthy hematopoietic cell lineages and improving the immune system defense. The autologous or allogeneic transplantation of UCB, BM, or MPB stem cells and their progenitors might be effectuated in combination with HDCT for numerous aggressive cancer forms to replace BM and blood-forming cells that have been destroyed by chemotherapy. AML and high-grade lymphoma are among the principal types of cancer that are usually treated with hematopoietic cell support as adjuvant therapy [16]. The different subtypes of AML appear to result from distinct mutations at the level of HSCs, the appearance of which may give rise to primitive leukemic stem cells (LSCs) possessing a specific phenotype, such as CD90$^-$, CD117$^-$, and CD123$^+$ [24]. These malignant LSCs, which are able to self-renew, might generate a heterogeneous AML cell population, thereby maintaining leukemic blasts [17]. Interestingly, it has been proposed that the maintenance of LSCs in quiescent status might contribute to their survival after chemotherapeutic treatment and leukemia relapse. Hence, the selective apoptosis of LSCs by using agents such as proteasome inhibitor MG-132 may constitute an adjuvant treatment for AML [18].

In addition, transplantation or mobilization of HSCs and their progenitors in systemic circulation is often used as immune support in combination with HDCT for the treatment of patients with certain highly aggressive solid tumors, and more particularly in advanced and metastatic stages of germinal cell tumors, retinoblastoma, myeloma, brain, lung, kidney, breast, and ovarian cancers [24]. However, the timing of the injection of HSCs during the disease and the number of grafted cells are among the major factors influencing the success of the engraftment and/or survival of patients. In this matter, the in vivo elimination of circulating tumor cells by purging prior to treatment may decrease the cancer progression in high-risk patients. Moreover, the ex vivo expansion of HSCs or the mobilization of HSCs from BM into the peripheral blood by using mobilizing agents such as G-CSF and AMD3100 might also lead to a great number of stem cells and their progenitors in bloodstream, thereby decreasing the recovery time after HDCT. The differentiated HSC-derived progenitors, such

as dendritic cells, which are among the most efficient cells of the immune system in presenting an antigen to helper/cytotoxic T lymphocytes, might also to be used as an adjuvant treatment in cancer immunotherapy to eliminate the neoplastic cells that express immunogenic antigens at their surface. Furthermore, UCB also contains a substantial amount of CD16$^-$/CD56$^+$ natural killer cells that might be expanded in the presence of IL-12 or IL-15 and that show a high rate of proliferation and cytotoxic effects against some cancers, particularly leukemia. In addition, the chemoprotection against myelotoxicity induced by HDCT may also be counteracted by genetic manipulations in HSCs conferring to their progenitors resistance to certain cytotoxic effects of drugs, such as the expression of MDR1 [16, 17].

Conclusion

These recent works in the field of stem cell biology have identified intrinsic mitogenic signaling cascades that are activated in mammary embryonic, fetal, and adult stem cells during the normal process of self-renewal and differentiation. These cellular events may also be implicated in the regenerating process after mammary gland injuries. Hence, this offers the possibility of differentiating these stem cell types into the specific mature cell lineages in vitro, ex vivo, and in vivo by using appropriate growth factors and cytokines for their use in basic research, as well as in transplantation for breast cancer. Several molecular targeting therapies may also be conceived by activation and blocking distinct developmental signaling cascade elements, such as BRCA1, EGFR, hedgehog, Wnt/β-catenin, and/or Notch pathways, which are frequently upregulated in cancer progenitor cells during the initiation and development of breast cancer.

X. Расси

ТЕРАПИЯ НАСЛЕДСТВЕННОГО РАКА ГРУДИ
С ПОМОЩЬЮ СТВОЛОВЫХ КЛЕТОК

Как наследственный, так и спонтанный рак груди может развиваться из-за нарушения регуляции путей самообновления нормальных стволовых клеток молочной железы. Совокупность протоонкогенов и супрессоров опухолей, которые контролируют пролиферацию раковых клеток, также регулирует самообновление стволовых клеток и, возможно, их старение. Ген *BRCA1* кодирует ядерный фосфопротеин, который

экспрессируется в целом ряде процессов, включая регуляцию стволовых клеток, репарацию повреждений ДНК, рекомбинацию, транскрипцию, убихитинирование, усиление клеточного цикла и регуляцию центросом. В настоящем исследовании сообщается о недавних достижениях использования предшественников зародышевых, плодных и взрослых стволовых клеток в терапии наследственного рака груди. Описаны варианты терапии с помощью молекулярного таргетинга путем активации и блокирования сигнальных каскадных элементов, таких как BRCA1, EGFR, hedgehog, Wnt/β-catenin и Notch pathways, которые часто регулируются в предшественниках раковых клеток в ходе инициации и развития рака груди.

X. Pacci

ТЕРАПІЯ УСПАДКОВАНОГО РАКУ ГРУДІ
З ДОПОМОГОЮ СТОВБУРОВИХ КЛІТИН

Як успадкований, так і спонтанний рак груди може розвиватися через порушення регуляції шляхів самооновлення нормальних стовбурових клітин молочної залози. Сукупність протоонкогенів та супресорів пухлин, які контролюють проліферацію ракових клітин, також регулюють самооновлення стовбурових клітин та, можливо, їх старіння. Ген *BRCA1* кодує ядерний фосфопротеїн, який експресується в ряді процесів, включаючи регуляцію стовбурових клітин, репарацію пошкоджень ДНК, рекомбінацію, транскрипцію, убіхітинування, посилення клітинного циклу і регуляцію центросом. В огляді наведено дані про недавні досягнення використання попередників зародкових, плідних та дорослих стовбурових клітин в терапії за допомогою молекулярного таргентинга шляхом активації та блокування сигнальних каскадних елементів, таких як BRCA1, EGFR, Wnt/β-catenin и Notch pathways, які часто регулюються в попередниках ракових клітин під час ініціації та розвитку рака груді.

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