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SALL4-STAT3 Relations In Primary And Metastatic Colon, Breast And Endometrial Cancer Cell Lines

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Özet

Amaç: Kanser gelişimi birçok moleküler mekanizmalar ile kontrol edilmektedir. Özellikle hücrenin embriyonik dönemdeki karakterini taşıyan, farklılaşması ve proliferasyonunda rol oynayan birçok yolak kanser gelişiminde de ortaktır. SALL4 kök hücrelerin transkripsiyonel düzenlenmesinde, pluripotansiyel ve kendi kendini yenilemesi özelliğinin korunmasında rol oynar. Embriyonik kök hücrelerin yanı sıra erişkin kök hücrelerde de gözlenmesi, sadece embriyonik dönemde değil erişkin dönemde de rolü olduğunu desteklemektedir. SALL4 ekspresyonu hem embriyonik hemde kanser gelişiminde rol oynayan birçok yolak ile birlikte gözlenmesine rağmen, SALL4 ekspresyonunun kontrolünün farklı dokularda hangi moleküller tarafından yapıldığı henüz açık değildir.

JAK/STAT sinyal yolağı hücrelerin proliferasyonunda ve farklılaşmasında rol oynayan bir yolaktır. Özellikle yolağın tümör gelişiminde de önemi ortaya konmuştur. Hücrenin farklı özelliklerinde rol oynayan STAT lardan STAT3, özellikle embriyonik kök hücrelerin kendilerini yenileme özelliklerini sürdürebilmeleri için gerekli olduğu saptanmıştır. Diğer STAT'ların aksine STAT3 gelişim için esansiyeldir. Bununla beraber SALL4 ile STAT3 ilişkisi bazı çalışmalarda gözlenmesine rağmen özellikle farklı kanser türlerindeki dağılımları çok ayrıntılı incelenmemiştir.

Çalışmada farklı kanser hücre hatlarında SALL4 ve STAT3 dağılımlarının tümör gelişimindeki rolleri ve özellikle primer ile metastatik kanser hücre hatlarındaki farklılıklarını ortaya konulması amaçlanmıştır.

Gereç ve Yöntem: Çalışmada primer (Colo-320) ve metastatik (Colo-740) kolon, primer (MCF-7), metastatik (M4A4) meme kanser hücre hatları ve endometrium adenokarsinoma hücre (CRL-1622) hatları kullanılmıştır. Colo-320, Colo-741, MCF-7, M4A4 hücreleri %10 FBS, %1 pen-streptamisin ve %1 L-glutamin ilavesi yapılmış RPMI-1640 kültür vasatında ve CRL-1622 hücre hattı ise %10 FBS, %1 pen-streptamisin ilavesi yapılmış DMEM-F12 kültür vasatında kültüre edilmişlerdir. Hücreler chamber slyatlara pasajlama işlemi yapıldıktan sonra SALL4 ve STAT3 dağılımları indirekt immunoperksidaz tekniği ile incelenmiştir.

Bulgular: Primer kolon kanseri hücre hattında (Colo-320) SALL4 immunoreaktivitesi kuvvetli pozitif iken, STAT3 immunoreaktivitesinin orta şiddette olduğu gözlendi. Metastatik kolon kanseri hücre hattında (Colo-741) ise SALL4 ve STAT3 immunoreaktivitelerinin benzer olduğu ve zayıf şiddette pozitif olduğu izlendi. Primer meme kanseri hücre hattında (MCF-7) SALL4 immunoreaktivitesi zayıf şiddette iken, STAT3 immunoreaktivitesinin kuvvetli pozitif olduğu saptandı. M4A4 hücre hattında ise SALL4 ve STAT3 immunoreaktiviteleri benzer olup orta şiddette idi. Endometrial adenocarcinoma hücre hattında (CRL-1622) SALL4 immunoreaktivitesinin STAT 3 immunoreaktivitesinden daha az olduğu saptandı.

Sonuç: Kolon kanserinde SALL4'ün, endometrial kanserlerde hem SALL4 hem de STAT3'ün, primer meme kanserlerinde ise özellikle STAT3'ün rol oynadığı gösterilmiştir. Sonuç olarak, farklı transkripsiyon faktörleri farklı kanser türlerini tetikleyebilir, o nednele, tedavilerde farklı kanser türleri için farklı olmalıdır.

Anahtar Kelimeler: Primer, metastatik, kanser hücre hattı, SALL4, STAT3

Abstract

Objective: Cancer development is controlled by many molecular mechanisms. Many pathways that play a role in the differentiation and proliferation of the cell, especially in the embryonic period of the cell, are also common in cancer development. SALL4 plays a role in transcriptional regulation of stem cells, preservation of pluripotency and self-renewal. Observation in embryonic stem cells as well as in adult stem cells supports the role not only in the embryonic period but also in the adult period. Although SALL4 expression is observed with many pathways involved in both embryonic and cancer development, it is not yet clear which molecules are responsible for the regulation of SALL4 expression in different tissues.

The JAK / STAT signal is a pathway that plays a role in the proliferation and differentiation of cells. Especially the role of pathway in the development of the tumor has been revealed. STAT3, which plays a role in different aspects of the cell, has been found to be essential for embryonic stem cells to maintain their self-renewal properties. Unlike other STATs, STAT3 is essential for development. However, although SALL4 and STAT3-related studies have been observed in some studies, their distribution in different types of cancer has not been examined in detail.

In the study, it was aimed to reveal the role of SALL4 and STAT3 distributions in tumor growth in different cancer cell lines and in particular differences in primer and metastatic cancer cell lines.

Methods: Primary (Colo-320) and metastatic (Colo-741) colon, primary (MCF-7) and metastatic (M4A4) breast cancer cell lines and endometrium adenocarcinoma cell line (CRL-1622) were cultured in RPMI-1640 (Colo 320, Colo 741, MCF-7, M4A4) or DMEM-F12 (CRL-1622) including 10% FBS, 1% penicillin-streptomycine and 2mM L-glutamin. SALL4 and STAT3 distributions were examined by indirect immunoperoxidase technique

Results: SALL4 immunoreactivity was strongly positive in the primary colon cancer cell line (Colo-320), whereas STAT3 immunoreactivity was moderately observed. In the metastatic colon cancer cell line (Colo-741), SALL4 and STAT3 immunoreactivity were similar and weakly positive. STAT3 immunoreactivity was found to be strongly positive while SALL4 immunoreactivity was weak in the primary breast cancer cell line (MCF-7). In the M4A4 cell line, SALL4 and STAT3 immunoreactivity were similar and moderate. SALL4 immunoreactivity in the endometrial adenocarcinoma cell line (CRL-1622) was found to be less than STAT 3 immunoreactivity.

Conclusions: SALL4 is thought to be involved in colon cancer, however, SALL4 and STAT3 in endometrial cancers, especially STAT3 in primary breast cancers could be responsible cancer cells properties. In conclusion, different transcription factors may trigger different cancer types, therefore the treatment could be differed according to cancer types.

Keywords: Primary, metastatic cancer cell lines, SALL4, STAT3

INTRODUCTION

Colorectal cancer is the second most common cancer both in men and women worldwide (1). Every year 140000 people are caught in the disease, and an annual average of 60000 people also died from this disease (1).

Breast cancer is a type of cancer begins in the breast cells. After lung cancer, breast cancer has the highest incidence in the world and one of every 8 women can be caught at a certain time in their life. Increase of breast cancer incidence has been detected since 1970s. This increase is indicated with western lifestyle. If breast cancer is detected early before spreading, the patient has a 96% survival chance (2). Endometrium cancer is a cancer of uterine inner membrane and, endometrial cancers are the most common type of cancer for women in the world (3).

Primary and metastatic cancer development and progression is controlled by numerous molecular mechanisms (2). Different molecular mechanisms may control the primary and metastatic cancer cells, in addition, these molecular mechanisms may have different effects on different patients, therefore, different responses are being taken in different patients. SALL4 protein, which has been found to play a role in the survival and differentiation of embryonic stem cells (4,5), has also been shown to be effective in the early stages of germ cells, and in particular distribution in the germ cell originated cancer cells has been studied (6). However, it is known that the molecules in the JAK/STAT pathway, which support cell proliferation and survival play a role both in embryonic development and cancer cell differentiation. However, association of SALL4 and STAT3 have not yet been identified in the different types of cancer.

Numerous cytokines and growth factors use Janus kinases (JAK) pathway during transmission of extracellular signals to the nucleus (7). JAK shows its effects with transcription activators (STAT-signal transducers and activators of transcription) intracellulary. This pathway, which is used by Type I and Type II cytokines, binds to cell surface receptors and activates signaling pathways, which have a controlling effect in the proliferation, differentiation and maintenance of the cell (8). However, mutations in the JAK / STAT signaling pathway, which also work together with other signaling pathways in the cell, trigger uncontrolled cell growth, differentiation, and other cellular events, as this pathway may be either permanently active or unable to be properly controlled (7,8).

In mammals, there are four types of JAKs; JAK1, JAK2, JAK3 and tyrosine kinaz2 (Tyk2) (Figure 1). Different JAKs are activated by different cytokines and each different JAKs induce different STATs in the cell (8). Binding cytokine to the receptor causes the receptor subunits to create the dimer and faster activation. The activation of JAKs provides tyrosine phosphorylation and it binds the many signalling proteins (STAT) to phosphotyrosine motifs (Src homology 2 (SH2) (Figure 1) (8). STATs are then phosphorylated to form dimers and transit to the nucleus to initiate gene transcription (8,9).

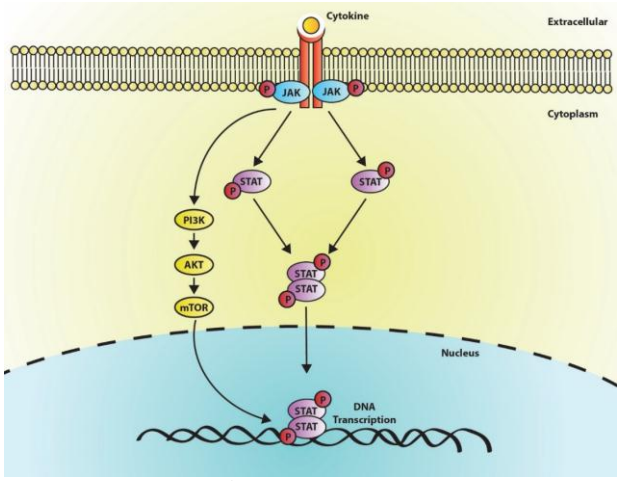

Figure 1: The activation of JAK/STAT signalling.

STATs are cytoplasmic transcription factors which control the genes of cell growth, differantiation and apoptosis (10-13). In mammals seven STATs are known; STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6 (10-13).

STAT3 has critical functions for the development of living beings (14). The signals of STAT3 are transmitted by cytokine receptors of IL-6 and IL-10 family members. STAT3 also plays a role in the anti-apoptotic signalling cascade (14). It has also been found that embryonic stem cells need STAT3 to survive their self-renewal (15). Contrast to other STAT's, STAT3 is essantial for mice early development (11). SALL4 is a member of Sal gen family which is a Zinc-finger transcription factor family member. The homologs of SALL4 has been identified in different species such as drosophila, xenopus, zebrafish, chicken, mouse and human (16). It is known that there are four Sal protein (SALL 1-4) both in mice and humans. SALL family members are responsible for many congenital disorders as well as being important for normal development (15). During embryonic development, SALL4 plays a role in formation of axis, brain, neural tube, pineal, somites, liver stem cells and expression of progenitor cells. Human SALL4 mutations cause Okihiro syndrome which is characterized with hearing, extremities and cardiac malformations (15). SALL4 plays a role in the regulation of Oct4 and Nanog which maintains the self renewal and pluripotency of embriyonic stem cells (Figure 2) (17-20).

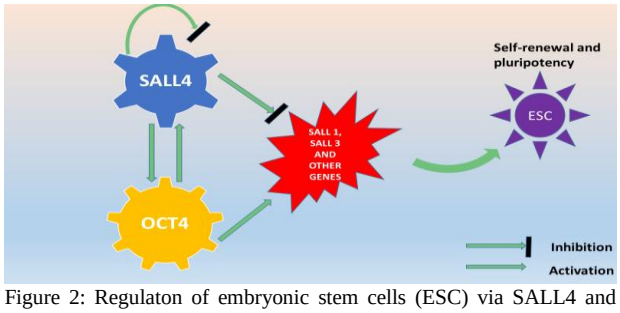

Figure 2: Regulaton of embryonic stem cells (ESC) via SALL4 and OCT4 expressions.

Recent studies have demonstrated that SALL4 is also effective in leukaemia development. It is shown that abnormal expression of SALL4 in acute myeloid leukaemia (AML) and precursor B-cell lymphoblastic leukaemia/ lymphoma (21), in addition the relation between SALL4 and chronic myeloid leukemia (CML) showed with other study (6,22).

Studies of the relationship between STAT3 and SALL4 are quite a few. In one of the studies, four binding sites are found with DNA sequence analysis that belongs to SALL4 gene promoter region and these are possible binding sites for STAT3. A significant reduction in SALL4 response to STAT3 was observed with the mutation of these regions, especially the region beginning with -199. Also in the same study, there was a dramatic decrease in SALL4 expression after suppression of STAT3 in MDA-MB-231 breast cancer cell line expressing STAT3 and SALL4 (4,5).

Both STAT3 and SALL4 play a role during normal and abnormal similar cellular properties, the relationship between them have not been evaluated. In this study, we aimed to investigate distributions of SALL4 and STAT3 in different cancer types such as; primary (Colo-320) and metastatic (Colo-740) colon, primary (MCF-7) and metastatic (M4A4) breast cancer and in endometrium adenocarcinoma cell line (CRL-1622) with immunohistochemical analyses.

MATERIALS AND METHODS

Cell culture

Primary human colon cancer cell cine (COLO-320, HTL95027, INTERLAB Cell Line Collection, Genova, Italy), metastatic human colon cancer cell line (COLO-741, HTL95008, Interlab Cell Line Collection, Genova, Italy), primary breast cancer cell line (MCF-7, HTB-22, ATCC), metastatic breast cancer cell line (M4A4, CRL-2914, ATCC) and endometrium adenocarsinoma cell line (CRL-1622, KLE, ATCC) were used. COLO-320 and COLO-741 human colon carcinoma cell lines in RPMI-1640 (F-1213, Biochrom, Berlin, Germany), MCF-7 and M4A4 cell lines in DMEM (BE12-614F), CRL-1622 cell line in DMEM-F12 (BE04-687F, Lonza) which were containing 10% fetal bovine serum (FBS, S 0113, Biochrom, Berlin, Germany), 2 mM L-glutamin (K0283, Biochrom, Berlin, Germany) and 1% penicillin-streptomycin (A 2213, Biochrom, Berlin, Germany) were cultured. They were cultured at 370C and 5% CO2 in air and the culture medium has been replaced every two days.

Immunohistochemistry

The cells were fixed with 4% paraformaldehyde for 30 min and were washed two times with phosphate buffer saline (PBS). They were then incubated 10 min with 0.1% Triton X-100 solution on ice for permeabilization and 3% H2O2 was applied for 5 min after washing with PBS. They were then treated with blocking solution (Invitrogen 859043) for an hour at room temperature and were incubated with primary antibodies; anti-SALL4 (sc-101147, Santa Cruz) and anti-STAT3 (sc-8019, Santa Cruz) for overnight at 4C0. The samples were washed with PBS and incubated with biotinylated rabbit anti-mouse seconder antibody (Invitrogen 859043) for 30 min. After washing with PBS streptavidin-hydrogen peroxidase (Invitrogen 859043) was added for 30 min. In order to determine the immunohistochemical reaction, diaminobenzidine (DAB, ScyTek, ACC125) was applied for 5 min. After washing with PBS, slides were stained with Mayer's hematoxylin and mounted with mounting medium. The intensity of immunlabelling were evaluated by the two investigators in different times with light microscopy (BX40, Olympus, Tokyo, Japan). The immunoreactivities were considered as negative (-), weak (+), moderate (++) and strong (+++).

RESULTS

Culture of Primary and Metastatic Cancer Cell Lines

Colo-320, primary colon adenocarcinoma cell line, which shows semi-adherent adhesive property was obtained from a 55-year-old female patient. Morphology is undifferentiated, round and monolayer cultured cells (Figure 3). Cells were passaged once a week and were found to tend to proliferate rapidly after passage.

Metastatic colon cancer cell line, Colo-741, was obtained from a 69-year old patient whose pelvic wall metastatic colo-adenocarcinoma. The cells were characterized adherent and epithelial shape (Figure 3) and they became confluent after 1 week of culture.

The breast cancer cell lines, MCF-7, was derived from 69-year-old female patient and after culturing, it showed adherent and epithelial shape properties (Figure 3).

The M4A4 metastatik breast cancer cell line was obtained from a 31-year-old female patient with epithelial shape and was observed fusiform structure (Figure 3). Both MCF-7 and M4A4 cells were 80% confluent after 7-10 days of culture.

The CRL-1622 cell line was obtained from a 64-year old female patient and was adherent characteristic endometrial adenocarcinoma cell line. The cells were epithelioid and reached 80% confluency after 14 days of culture (Figure 3).

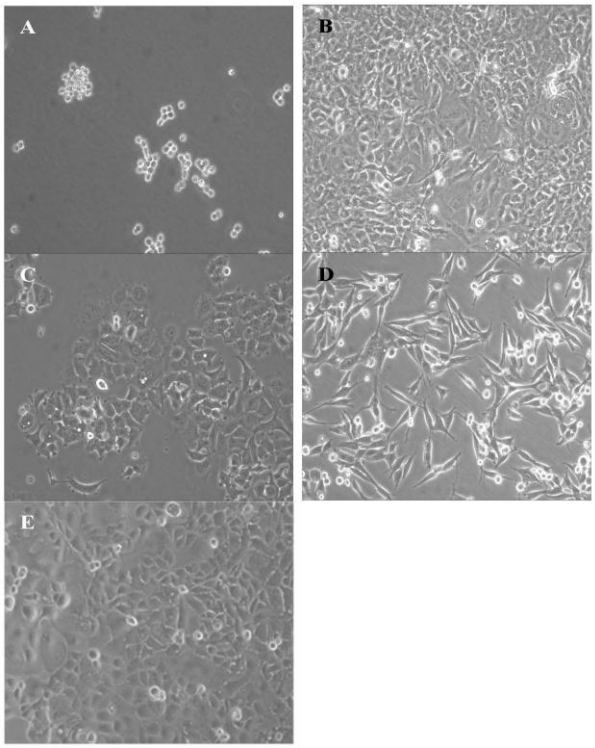

Figure 3: Cell culture images of Colo-320 (A), Colo-741 (B), MCF-7 (C), M4A4 (D) and CRL-1622 (E) cells. X400

Distributions of SALL4 and STAT3

SALL4 immunoreactivity was strongly positive in the primary colon cancer cell line (Colo-320), while STAT3 immunoreactivity was moderate (Figure 4, Table 1). In the metastatic colon cancer cell line (Colo-741), SALL4 and STAT3 immunoreactivities were similar and weakly positive (Figure 4, Table 1). STAT3 immunoreactivity was found to be strongly positive while SALL4 immunoreactivity was weak in the primary breast cancer cell line (MCF-7) (Figure 4, Table 1). While SALL4 and STAT3 immunoreactivities were similarly detected in M4A4 cells, moderate immunoreactivity was detected (Figure 4, Table 1). SALL4 immunoreactivity in the endometrial adenocarcinoma cell line (CRL-1622) was found to be less than STAT3 immunoreactivity (Figure 4, Table 1).

	SALL 4	STAT 3
Colo-320	++++	++
Colo-741	+	+
MCF-7	+	+++
M4A4	++	++
CRL-1622	+	++/+

Table 1: Intensity of SALL4 and STAT3 in Colo-320, Colo-741, MCF-7, M4A4 ve CRL-1622 cell lines.

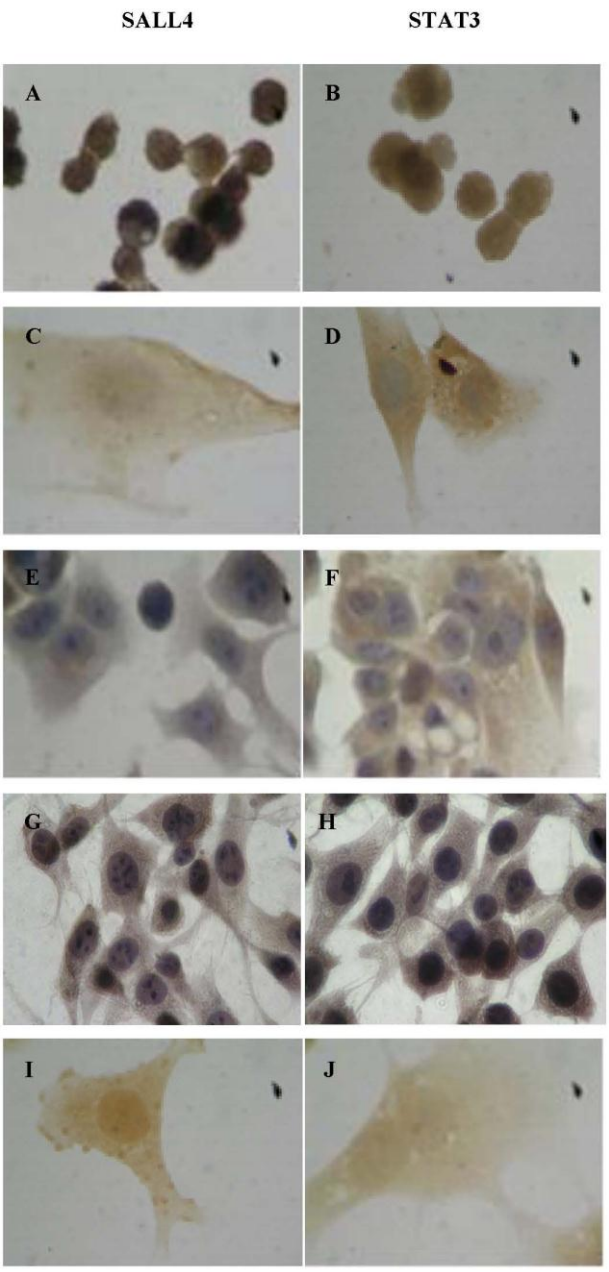

Figure 4: Immunohistochemical analyses of SALL4 (A, C, E, G, I) and STAT3 (B, D, F, H, J) in Colo-320 (A, B), Colo-741 (C, D), MCF-7 (E, F), M4A4 (G, H) ve CRL-1622 (I, J) cell lines. X1000.

DISCUSSION

STAT proteins are activated with various growth factors, cytokines and other ligands to work in basic cell function like cell proliferation, growth, differentiation, inflammation and apoptosis (8,9). In many types of cancer, it has been shown that STATs have some abnormal activation such as cellular transformation and oncogenic potential (23). Cytokines and growth factors phosphorylate the JAK family members for activating STAT proteins. After that STAT proteins phosphorylate specific tyrosine kinase residues for initiating the transcription (24,25). The activation of STAT3 has been observed in many cancers such as breast, leukemia, lymphoma, lung and thyroid (26,27). Therefore, drugs that inhibit the activation of STAT3 have come into question in treatments, and it is emphasized that STAT3 expression or control mechanisms may used as an early and / or late markers. Especially in colon pathology,

inflammatory diseases are important for creating cancer trigger, during this period secreted cytokines play a role because of the communication between STAT3 and IL6 so that they effect JAK / STAT pathway. For these reasons in primary colon and metastatic cancer types investigate the effects are clinically important (23- 31). SALL4, which plays an important role during embryonic development and especially in the differentiation of germ cells, is also important in embryonic stem cell homeostasis (4,5,6,21). Especially there are studies supporting the reduction of SALL4 expression leading to apoptosis (20). Above all, providing protection for the stem cell character and the importance in cancer cells SALL4 one of the new areas of research. In our study, SALL4 immunoreactivity was detected higher in primary colon cancer comparing to other primary tumors, therefore, it is suggested that the SALL4 immunoreactivity could be important continuity of the primary colon cancers cells into stem cells. However, eventhough STAT3 expression intensity was not observed as much as SALL4, moderate intensity observation suggested that STAT3 may have a role in transcription of primary colon cancer cells. Both SALL4 and STAT3 were expressed in primary endometrial cancer cells, but SALL4 intensity was weaker then STAT3. In primary breast cancer cells, STAT3 is a particularly important transcription factor because of abundant expression than than SALL4. In the metastatic breast cancer cell line, both SALL4 and STAT3 immunoreactivity were similar, therefore, both proteins may control and progression of metastatic property. In primary and metastatic cancers cell characteristics include similar and different factors that promote cellular proliferation and / or transcription in the cell. In particular, during treatment of the individual mutations as well as cell-specific factors, or cells after differentiation to change the factors affecting the homeastasis be considered the treatment of choice in achieving effective treatment protocols.

CONCLUSION

In conclusion, SALL4 in colon cancer, SALL4 and STAT3 in endometrial cancer, STAT3 in primary breast cancer play a role cancer maintenance. Our results showed that the different cancer types may trigger different transcription factors, therefore different cancer type treatment has to be assesed according to these factors.

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