We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Splicing Factors in Breast Cancer: Drivers of the Breast Tumor Fate

Felipe A. C. da Luz, Paula C. Brígido,
Alberto S. Moraes, Rogério A. Araújo and
Marcelo J. B. Silva

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/66162>

Abstract

Splicing is a critical step in gene expression, responsible for the excision of introns, producing the mature form of mRNA. Also, the possible arrangements of exons enlarge the proteome in 80%, enabling one gene to encode more than one protein isoform, thus increasing proteome. Growing data show deregulation of splicing events in cancer, being breast cancer the most studied. This aberrant pattern of splicing has an important role in breast tumor progression. These alterations are mainly caused by misexpression of some critical alternative splicing factors. The behavior of these splicing factors is implicated with important clinical features, such as chemoresistance, aggressiveness, and also metastases. In this chapter, the role of five splicing factors is discussed in the light of relevant data about *in vitro*, *in vivo*, and *ex vivo* studies to construct a representative scheme of their behavior in breast cancer progression. Although the presented five splicing factors have important role in breast cancer, only three of them (ESRP1, RBFOX2, and SRSF1) have a more prominent role in tumorigenesis and tumor progression. These concepts will elucidate their role in tumorigenesis and a prospective use as biomarkers in breast cancer.

Keywords: breast cancer, aberrant splicing, tumor progression, splicing program, splicing factors

1. Introduction

1.1. Breast cancer: subtypes and epidemiology

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among women worldwide, with an estimated 1.7 million cases and 521,900 deaths in 2012 and accounts for 25% of all cancer cases and 15% of all cancer deaths among women [1]. The USA government expected 232,670 new cases of breast cancer in 2014, representing 29% of all cancers in women. Also, breast cancer represents 15% of all women cancer deaths [2].

If diagnosed in the initial stage, breast cancer is curable. However, at advanced stage, it is almost incurable. Actually, late diagnosis is one of the main factors that contribute to the poor prognosis of breast cancer patients [2].

Clinically, this heterogeneous disease is divided in three basic therapeutic categories: hormonal receptor-positive (ER/PR-positive; luminal A and luminal B), the most common and numerous, with several prognostic tests for hormonal-based therapy-treated patients; HER2/neu+ or ERBB2⁺, with poor prognostic, but with target therapy, there is an improve in survival; and the triple-negative breast cancers (ER-negative, PR-negative, and HER2/neu-negative), with seven subtypes, the majority is aggressive, its treatment is more limited principally with chemotherapy, and has an incidence associated with mutated BRCA1 lineage or African ascendancy [3]. Another category, recently classified, was denominated as claudin-low, which is also triple-negative, but with low expression of proteins of cell-to-cell junctions, especially tight junctions, making it a highly infiltrating tumor [4].

1.2. Spliceosome and splicing: molecular basis of a critical event

All eukaryotic genes contain intragenic regions (introns), that usually not encode expression sequence to produce proteins, and expression regions (exons), that are the responsible for encoding expression sequence to produce proteins. Therefore, when expressing a gene, it is important to remove (excising) introns and the constitutive splicing is the event responsible for this event. In addition, approximately 95% of human genes encodes more than one protein isoform. This is achieved by differentially splicing the exons of the gene, called alternative exons, by alternative splicing. Importantly, alternative splicing is responsible for about 80% of gene variability [5].

Splicing is performed by the spliceosome, a core complex formed by five subcomplexes of snRNPs (small nuclear ribonucleoproteins), called U1, U2, U4/5 (always present as a bi-sub-complex), and U6 (U from **uracil** rich), and they participate in different steps during splicing. Several spliceosome-associated proteins, named splicing factors, coordinate the constitutive and alternative splicing. Alternative and constitutive splicing operate through a combination of positive and negative signals, called silencers and enhancers (*cis*-acting signals), present in the pre-mRNA (premature RNA) that are recognized by several splicing factors (*trans*-acting factors). In addition to *cis*-acting signals and *trans*-acting factors to splicing be exerted, the integrity of DNA and epigenetic changes (e.g. histone post-translational modifications) are important for physiological splicing as they can alter, or dictate,

transcription rate and interaction of a splicing core complex and splicing factors, with the transcript and nucleosome [6, 7].

The most studied alternative splicing factors are the proteins of the SR (serine/arginine rich proteins) family, and hnRNP (heterogeneous nuclear ribonucleoproteins). These two types of proteins have RNA recognition motifs (RRM) and other domains that allow protein-protein and RNA-protein interactions during splicing [5].

1.3. Understanding the splicing factor stoichiometric relationship

As mentioned above, splicing factors can interact with proteins and RNA. Each splicing factor can recognize a different RNA sequence, therefore each splicing factor participates in the pre-mRNA excision (splicing) of a specific group of genes. Thus, the action spectra of each splicing factor are limited [5].

Many splicing factors are involved in the alternative splicing of a same pre-mRNA. For example, *FGFR2* (fibroblast growth factor receptor 2 gene) pre-mRNA has a UGCAUG sequence in the exon IIIc; this sequence is recognized by the splicing factor RBFOX2 in the exon IIIc, but, upstream to exon IIIc exists another sequence (CUGGGA) the SRSF1 splicing factor. The resulting interaction will dictate the resulting isoform: the IIIc (mesenchymal) or IIIb (epithelial) transcript isoform of *FGFR2*. Notwithstanding, the splicing factor hnRNP H/F recognizes the GGG sequence that is within the sequence (CUGGGGA) that SRSF1 recognizes, and inhibits the alternative splicing (exon IIIc inclusion). In addition, RBFOX2 interacts with hnRNP H/F. Thus, an interactive network is formed by these four splicing factors in a stoichiometric and competitive manner, depending on the expression levels of each splicing factor. Also, these splicing factors that compete by the same RNA region (SRSF1, hnRNP F/H) bind with different affinities [8].

Not only is the stoichiometric relationship important to dictate production of transcript isoform production but the localization of the splicing factor network is critical to alternative splicing. In the cited example (*FGFR2* alternative splicing), when the RBFOX2-hnRNP H/F network associates downstream of the exon IIIc, the splicing factors act toward alternative splicing, but they act as alternative splicing suppressors when associated upstream of the exon [8].

In summary, the association site of splicing factors determines the transcript destiny (constitutive, alternative or even aberrant, truncated, or degraded) [5]. However, how the splicing pattern of a cell is governed? Some studies elucidated whether splicing program is specific for each cell. The knowledge about this matter may help to understand why cancer cell presents such an aberrant splicing pattern.

1.4. Regulating the expression and activity of splicing factors

The relationship between splicing factors is mainly stoichiometric, and the expression levels of the splicing factors determine the expression of a dominant isoform of a transcript. Thus, one important question is raised: how the expression and activity of the splicing factors

determined? Like other proteins by specific stimuli. For example, the activation and activity level of SR splicing factors are determined by their phosphorylation state (hypo- or hyperphosphorylation) [14, 15], and the splicing activity depends on proper *de novo* phosphorylation and dephosphorylation [16–18]. One well-known stimulus is the activation of Ser/Arg-rich protein kinase 1 by AKT [19]. The AKT signaling pathway is activated by epidermal growth factor signaling [20]. Another example is the phosphorylation of SAM68 by MAPK, which regulates the CD44 alternative splicing [21]. Similar stimuli are important for regulating the levels of splicing factors and for noncoding RNA, mainly miRNA [22–25]. Therefore, regulation of splicing factor expression and activity, according to their own properties, is similar to other genes and proteins.

1.5. Understanding the splicing programs in physio(patho)logical contexts

The knowledge about the cellular splicing pattern (splicing program), that is, the production of cell-specific transcript isoform, consequently cell-specific protein isoforms, help to understand cell programming and fate, and, by extension, tumor fate.

The splicing pattern of pluripotent cells differs from a differentiated cell. During differentiation, the transcripts are differentially spliced, generating different isoforms (splicing shift). In addition, some splicing factors are differentially expressed. Nevertheless, induction of pluripotent cells from adult cells reverts the splicing program features. Also, the ectopic regulation of the splicing factors that govern the splicing shift results in an effectively splicing program shift [9].

These characteristics, that is, the regulation of cellular differentiation-specific splicing program by specific splicing factors, also occur in tissue-specific cells. It is now understood that the major cell types (epithelial, endothelial, and mesenchymal cells) have a common splicing program among these cell types of different organs, but a different splicing program among these cell types, and at least one main splicing factor governs each splicing program (ESRP1, PTBP1, and RBFOX2 for epithelial, endothelial, and mesenchymal cells, respectively). Notwithstanding, the cell type-specific splicing programs observed in these cell types may be controlled by a balanced expression of antagonist splicing factors, as well as antagonistic interactions between these three main splicing factors (ESRPs, RBFOX2, and PTBP1) [10].

Another important characteristic in physiologic splicing program regulation is the splicing shift and balance in cellular reprogramming, such as EMT (epithelial-to-mesenchymal transition). When EMT is triggered in epithelial cells, which have a high epithelial splicing program/low mesenchymal splicing program ratio, a balance is created by a splicing program switch. These cells express higher levels of RBFOX2 and have several splicing features similar to mesenchymal cells [11].

In carcinoma cells, a similar pattern is observed. These cells commonly have an epithelial splicing program/mesenchymal splicing program ratio resembling their phenotype, that is, a cell with lower epithelial features and high mesenchymal features has a lower epithelial splicing program/mesenchymal splicing program ratio, that is, their splicing program prone to a mesenchymal splicing program [11]. In addition, growing data point to a common splice

program in cancer, considering its cellular type (epithelial, endothelial, or mesenchymal), despite its origin, with the main governing splicing factors as in healthy cells [12]. Understanding these common splicing program features shared among tumors allows us to predict tumor behavior during tumor progression in breast cancer according to their differentiation state.

1.6. Epithelial-to-mesenchymal transition (EMT)

Epithelial-to-mesenchymal transition (EMT) is the most important mechanisms that allow epithelial malignant cell to migrate, leading to metastases. During EMT, epithelial cells that are naturally attached to their tissue lose their epithelial markers and gain mesenchymal characteristics, being able to move away from tissue and migrate to a distant site, where they settle and return to an epithelial state by the reverse event mesenchymal-to-epithelial transition (MET) [12–14]. Several transcription factors regulate EMT, and other, different, regulate MET. As example, proteins of the Twist, Snail, and Zeb families, the well-known transcription factors of EMT, recognize specific DNA sequences (regulatory regions) near the promoters of genes, repressing genes of epithelial markers. Also, these transcription factors are involved in the expression of genes related to extracellular matrix degradation and migration [13, 14].

Important, misregulation of splicing factors has been observed during this event, and they are involved with a switch to the production of mesenchymal isoforms of several genes [11, 15–17].

1.7. Aberrant splicing in breast cancer

In cancerous cells, it is possible to observe an aberrant, thus different, pattern of splicing of several genes, even without DNA sequence change (mutations) or epigenetic changes [16, 18]. Actually, these alterations are due to the expression of misregulated splicing factors.

Several studies observed the misregulated expression of splicing factors in cancer, mainly breast cancer, although suggesting that the aberrant splicing is caused by an unbalanced stoichiometric relationship of splicing factors, compared to normal cells, leading to malignant features. Thus, many studies summarized the misregulated splicing factors observed in cancer and/or the consequences of aberrant splicing, including breast cancer [18–20]. For example, upregulation of RBFOX2, SRSF1, SF3B1, hnRNP A1, hnRNP F, and hnRNP H and downregulation of ESRPs are involved with tumor aggressiveness in breast cancer and other cancers [9, 11, 17, 21–38].

The growing evidence of splicing misregulations in cancer prompted several studies to understand the impact in splicing by these misregulations.

2. Misregulated splicing factors: directing the tumor fate

Aberrant splicing program in cancer cells is altered in comparison to healthy cells, but still is governed by splicing factors. In breast cancer, aberrant splicing is intricate with alternative and constitutive splicing factors. However, only the most relevant and most studied splicing factors

in breast cancer are presented to understand the splicing behavior during breast cancer tumor progression. These splicing factors are representative of main types: SF3B1, component of spliceosomal core complex; SRSF1, a member of SR family; ESRP1 and ESRP2, alternative splicing factors with main role in tissue-specific (epithelial) splice program; and RBFOX2, the main alternative splicing factor that governs tissue-specific (mesenchymal) splice program.

Complementary data related to these splicing factors in other cancers will be presented for a greater understand of the tumor progression process in breast cancer.

2.1. SF3B1

SF3B1 (also known as Sap155, Sf3b155), from the SF1 complex, is responsible for the spliceosome core assembly in the pre-mRNA [5]. It is a constitutive splicing factor and is critical to the event. The SF3B1 protein complex is responsible for mis-spliced mRNA retention, performing quality control, which is the inspection of the intron excision and exon junction [39].

Despite the fact that this splicing factor is mostly studied in hematologic malignances [40] and its role in solid tumors is still being elucidated [41]. Some breast cancer patients showed a mutation in SF3B1 that was associated with the alternative splicing of key genes in ER-positive breast cancer, such as genes involved with cell metabolism, cell cycle, cell motility, protein degradation, apoptosis, and other cell events [42].

One extensive study showed upregulation of SF3B1 and SF3B3, associated with endocrine resistance in breast cancer samples, as well as two important correlations: in ER-positive breast cancer, the aggressiveness was associated with higher expression of SF3B3, while the aggressiveness of ER-negative breast cancer was associated with lower expression of SF3B1. Thus, researchers were able to correlate the levels, high or low, of SF3B3 and SF3B1, respectively, with prognosis in breast cancer patients, according to their ER status [32].

Although some studies reported impairment in the expression levels of constitutive splicing factors, such as SF3B1, other studies observed different behaviors. In one study, overexpression of some core complex splicing factors was observed in MYC-overexpressing breast cancer. Inhibition, or knockdown, of SF3B1 or BUD31, another component of the splicing core complex, but not of other core complex splicing factor, led to impaired tumor growth, reduced metastases, and apoptosis resistance abolishment in MYC-overexpressing breast cancer, without the substantial effect in normal cells. Studies also found that the overexpression of these splicing factors is related to the increased transcription rate caused by MYC-overexpression [33].

In brief, deregulation of SF3B1 as well as **constitutive** splicing factors is implicated with cell transcription rate rather than an oncoprotein *per se*.

2.2. SRSF1

SRSF1 is involved, additionally to splicing, with mRNA transport (nuclear exporting) and translation (in association with eIF4E) [43]. It has one SR domain, in which the main regulatory function occurs, and two RRM domains that interact with RNA and other splicing factors [44].

The important role of SRSF1 was intensively analyzed in several studies. Overexpression of SRSF1 in mammary epithelial cells conferred apoptosis resistance by alternative splicing of apoptosis proteins BIM and BIN, high proliferative rate, invasion of skin and muscle, slight necrosis, high angiogenesis, and well-defined borders *in vivo* [30]. Upregulation of SRSF1 was responsible for conferring cell motility in several human adenocarcinoma cell lines by regulating alternative splicing of RON (a tyrosine kinase receptor), generating an alternative isoform that confers motility properties [31]. Similar results were obtained by other researchers [25]. Also, chemotherapy resistance was observed due to SRSF1 overexpression concomitant to EMT [45]. The role of SRSF1 upregulation in tumorigenesis was corroborated by other studies. Knockdown of SRSF1, or one of its products, in SRSF1-overexpressing cancer cells reverted the tumor features to a normal phenotype [25, 46].

Regarding the activity of SRSF1, very important results have been obtained. In one study, it was observed a differential expression of SRSF1 in cancer cell lines: it is highly expressed in the mesenchymal-like cells (low-density culture) when compared to the epithelial-like cells (high-density culture). The difference in the expression levels is related to an EMT alternative splicing profile: EMT cells produce a full-length mRNA of *SRSF1*, while epithelial cells produce an mRNA with a premature stop codon, leading to degradation of *SRSF1* mRNA. The main cause of that striking difference was analyzed: mesenchymal-like cells have highly phosphorylated ERK1/ERK2 pathway proteins compared to epithelial-like cells, and inhibition of the phosphorylation status in this pathway led to a hypophosphorylated status of Sam68, another splicing factor that mediates the splicing of *SRSF1* results in the production of an isoform with a premature stop codon, decreasing SRSF1 levels [47]. Thus, SRSF1 levels depend on signaling pathways in cancer cells too, and extracellular signals that led to EMT are also responsible for SRSF1 upregulation. This is corroborated by inhibition of proteins' intricate in important pathways that led, or sustain, EMT. For example, inhibition GSK3 kinase, as well as knockdown of AKT and GSK3beta kinase, led to SRSF1 downregulation, with a loss of apoptosis resistance and colony formation impairment [48]. In addition, inhibition of SRK1, a kinase of several SR proteins, led to reduced tumor growth *in vivo* through inhibition of angiogenesis [49]. Therefore, the activation of SR splicing factors by phosphorylation is crucial in the tumor context.

SRSF1 upregulation was observed not only in *in vitro* and *in vivo* models, but also in *ex vivo* samples. SRSF1 upregulation is observed in malignant tissue, but not in nonmalignant lesions [49]. SRSF1 upregulation was observed in several tumors, if compared to normal surrounding tissue, concomitant with antiapoptotic isoform of *BIN1* and oncogenic isoform of transcript targets of SRSF1 were observed in *in vitro* studies [25]. Moreover, SRSF1 upregulation was found to be correlated with tumor invasiveness only in some malignant lesions [45]. The tumorigenic role of SRSF1 is corroborated by the synergistic correlation between the oncogene *MYC*. A high expression of *SRSF1* occurs significantly more often in tumors that overexpress *MYC*, and positively correlated with a high histological grade compared to low *SRSF1* and/or low *MYC*-expressing breast tumors [30].

In summary, SRSF1 is an oncoprotein *per se*, as its overexpression causes tumor promotion, EMT, aggressive phenotypes, and chemoresistance. Nevertheless, signaling pathways

directly regulate its activity and expression level. In addition, it is intricately related to the cell transcription rate, as seen in the overexpression of *MYC* in breast cancer.

2.3. ESRP1 and ESRP2

Epithelial splicing regulatory proteins (ESRPs) 1 and 2 participate in the epithelial-specific splicing program, downregulated during EMT [50]. ESRP1 knockout is lethal in embryos, and several developmental genes are regulated by these splicing factors [51].

As ESRPs are involved in the splice program in epithelial cells and play an important role in EMT. EMT transcription factors downregulate ESRPs, impacting the production of several protein isoforms coded by different genes, such as *FGFR2* and *CD44*, as well as adhesion molecules, surface receptors, and cytoskeleton [50, 52–56], independently of the stimuli that trigger EMT [34, 36, 57], resulting in predominance of mesenchymal splice program, mainly by ESRP1 downregulation [17, 34]. Otherwise, ESRP1 overexpression abrogates EMT [36, 54, 56, 58, 59], and the inverse phenomenon—the induction of MET—upregulates ESRP1 and reverts EMT [60].

The intricate role and behavior of ESRP1 in EMT seem to be orchestrated by upstream regulators, and indeed it is. ESRP1 expression is directly inhibited by some EMT transcription factors that recognize specific sequences near ESRP1 promoter, repressing its expression during EMT [54, 59].

Although all data appoint to ESRP1 downregulation in tumorigenesis and tumor progression, ESRP1 overexpression can contribute to the tumoral process and metastasis too [61]. However, ESRP1 downregulation is more expected in tumor progression than its upregulation. ESRP1 upregulation can be found within tumor lesion and can correlate with tumor progression, but ESRP1 downregulation is observed in the invasive front, mainly in invasive tumors with EMT features [34]. That phenomenon can be found concomitant with downstream products of ESRP1, like *FGFR2-IIIc* expression in function of ESRP1 downregulation, but not necessarily with metastases [37]; also can be found in poorly differentiated cancer and correlate with poor prognosis [36]. Also, ESRP1 downregulation is intricate with advent of cancer stem-like cell features in breast cancer [55].

In brief, upstream effectors that dictate cell phenotype and behavior, mainly EMT/MET-TFs, also regulate ESRP expression, the main **alternative** splicing factor that governs the epithelial splice program. Other transcription factors, such as stem transcription factors, may also regulate ESRPs, as ESRP upregulation was observed concomitantly with stemness. The low expression level of ESRPs led to the production of alternative transcript isoforms of key genes involved with cell metabolism, cell cycle, motility, invasiveness, and robustness.

2.4. RBFOX2

RBFOX2 (also known as Fox2 and Rbm9, and formerly known as the repressor of tamoxifen transcriptional activity—RTA) is a tissue-specific splicing factor [11]. It is directly associated with the production of alternative protein isoforms and rarely to constitutive isoforms [62].

The first study that described RBFOX2 as an RRM-containing protein observed that it inhibits the partial ER β agonistic activity of tamoxifen through this domain. Moreover, this splicing factor is intricately related to the repression of ER β , PR (progesterone receptor), and GR (glucocorticoid receptor) activities. With these data, it is inductive to think that RBFOX2 is a repressor of activity in the steroid receptor family [63]

The role of RBFOX2 in EMT has been partially elucidated and has been intensively studied in breast cancer. Different cancer cell lines have high expression of RBFOX2 [11]. This splicing factor is associated with a mesenchymal identity, since depletion of RBFOX2 in breast cancer with mesenchymal features induced MET, thus reverting EMT [11, 17]. Similar events occur in mouse breast cancer cells. EMT causes Rbfox2 (mouse homolog of RBFOX2) overexpression, and abolishment of EMT reestablishes the expression levels of Rbfox2. Also, Rbfox2 is important to maintain invasive features [15].

RBFOX2 expression and splicing activity are regulated by some EMT transcription factors, leading to a similar splicing pattern to that of breast cancer cells, which is associated with the expression of distinct isoforms of several genes that govern EMT [17]. RBFOX2 expression is altered in some cancer in comparison to their normal counterparts [64]. In addition, RBFOX2 has an important role in the overall splicing pattern in breast cancer [65] and is more expressed in claudin-low and basal-like cancers than in luminal A and B, leading to the aberrant inclusion of alternative exons observed in cancer [35]. These aggressiveness of these breast cancer subtypes (claudin-low and basal-like) could be partially explained by the resultant splicing events of RBFOX2 upregulation [35].

In summary, RBFOX is the primary **alternative** splicing factor that governs the mesenchymal splice program, mainly EMT/MET-TFs. Other transcription factors, such as stem transcription factors, may regulate RBFOX2. The expression level of RBFOX2 leads to the production of alternative transcript isoforms of key genes involved with cell metabolism, cell cycle, motility, invasiveness, and robustness.

3. Lessons from alternative splicing misregulation

Albeit few splicing factors were presented in the last section, though the most important splicing factors, to date, in breast cancer, the understanding of their role in tumor promotion and progression in breast cancer allows us to construct and predict the behavior of these splicing factors, according to clinical features and molecular subtype. Several splicing markers can discriminate ER⁺ from ER⁻ breast cancer and correlate with a tumor grade, demonstrating that the ER status impacts the splicing program [11, 35, 56, 66, 67]. Therefore, the splicing factors play a role in a key and basic event (alternative splicing) that dictates several features and phenotype of cells.

The main impact of splicing observed in cancer is due to alternative splicing factor misregulation rather than the constitutive splicing factor, as mentioned in the previous section. In addition, not all alternative splicing factors have an expressive role in alternative splicing, but

only few main splicing factors. ESRP1 and RBFOX2, splicing factors that govern splicing programs, are responsible for general aggressive phenotypic features of breast cancer, and SRSF1 plays a critical role in breast cancer tumorigenesis and malignancy. These findings allow us to predict general features of breast cancer according to the behavior (expression) of these splicing factors. Current data about these three splicing factors in breast cancer let the construction of a hypothetical scheme of well- and poor-differentiated breast cancer, starting from tumor promotion [30, 43], following tumor progression [15, 25, 30, 31, 43, 45–47, 52], metastasis, and acquired resistance (clone selection) [11, 15, 31, 34, 38, 47, 50, 53, 54, 61], regarding the behavior of these splicing factors (**Figure 1**). Summarizing these findings in one figure opens a door to an important practical use of that knowledge: a potential power as diagnostic and prognostic markers.

Figure 1. SRSF1, ESRP1, and RBFOX2 participation in tumor progression. Expression patterns of splicing factors according to the pathological status of tumors. Well-differentiated tumor (upper side): according to the literature, well-differentiated tumors express higher levels of ESRP1 and SRSF1 and lower levels of RBFOX2 compared to normal surrounding tissue, and these characteristics are most striking with tumor progression. However, the invasive front has an inverse pattern that is similar to EMT cells. The majority of metastatic cells that originate from these tumors do not have CSC features. Poorly differentiated tumor (lower side): according to the literature, poorly differentiated tumors express higher levels of RBFOX2 and SRSF1 and lower levels of ESRP1 compared to normal surrounding tissue, and these characteristics are most striking with tumor progression. As these cells have EMT features (mesenchymal-like), the invasive front is derived from the tumor pool in the primary niche. The majority of metastatic cells that originate from these tumors have CSC features. These two tumors have two fates in response to chemotherapy: cure and resistance. Although well-differentiated tumors are more sensitive to chemotherapy, with a higher cure index, resistant clones in the primary tumor niche, mainly EMT cells, can be selected and expanded, originating a poorly differentiated tumor; the metastatic cells and secondary tumor cells are cleared with chemotherapy, but CSCs, when present, are selected and expanded. Resistant cells (CSCs) express even higher levels of RBFOX2 and SRSF1 and even lower levels of ESRP1 with an EMT phenotype.

4. Concluding remarks

Although the prognostic and diagnostic value of splicing factors is not well understood, some studies have shown that an expression pattern of some splicing factors is observable in breast cancer. In addition, analysis of splicing factors related to splicing programs help to understand other processes involved in tumor progression, like aggressiveness. Also, other splicing factors are related to chemoresistance and hormonal status, mainly estrogen. Thus, the behavior of splicing factors could be used, at least, as prognostic markers for breast cancer and could help to choice, or direct, therapy in a near future.

Acknowledgements

We gratefully acknowledge financial support from the Brazilian Agencies CAPES, FAPEMIG and MCT/CNPq, and NUPPEC-Cancer Hospital of Uberlândia/Grupo Luta pela Vida.

Abbreviations

ESRP1	Epithelial splicing regulatory protein 1
RBFOX2	RNA binding protein fox-1 homolog 2
SRSF1	Serine/arginine rich splicing factor 1
SF3B1	Splicing factor 3B subunit 1
PTBP1	Polypyrimidine tract binding protein 1
ER	Estrogen receptor
PR	Progesterone receptor
ERBB2	ERB-b receptor tyrosine kinase 2
FGFR2	Fibroblast growth factor receptor 2
hnRNP	Heterogeneous ribonucleoprotein particle
MAPK	Mitogen activated protein kinase
EMT	Epithelial mesenchymal transition
MET	Mesenchymal epithelial transition
ERK	Extracellular signal-regulated kinases

Author details

Felipe A. C. da Luz¹, Paula C. Brígido², Alberto S. Moraes³, Rogério A. Araújo⁴ and Marcelo J. B. Silva^{1*}

*Address all correspondence to: majbsilva@gmail.com

1 Laboratório de Osteoimunologia e Imunologia dos Tumores, Instituto de Ciências Biomédicas (ICBIM), Universidade Federal de Uberlândia (UFU), Uberlândia, MG, Brazil

2 Laboratório de Tripanossomatídeos, Instituto de Ciências Biomédicas (ICBIM), Universidade Federal de Uberlândia (UFU), Uberlândia, MG, Brazil

3 Department of Biologia Celular, Histologia e Embriologia, Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia, Uberlândia, MG, Brazil

4 Hospital do Câncer, HC, UFU, MG, Brazil

References

- [1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin.* 2015;2:87–108. Doi: 10.3322/caac.21262.
- [2] Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin.* 2014;1:9–29. Doi: 10.3322/caac.21208.
- [3] Schnitt SJ. Classification and prognosis of invasive breast cancer: from morphology to molecular taxonomy. *Mod Pathol.* 2010; 23:S60–64. Doi: 10.1038/modpathol.2010.33.
- [4] Network TCGA. Comprehensive molecular portraits of human breast tumours. *Nature.* 2012;7418:61–70. Doi: 10.1038/nature11412.
- [5] Wahl MC, Will CL, Luhrmann R. The spliceosome: design principles of a dynamic RNP machine. *Cell.* 2009;4:701–718. Doi: 10.1016/j.cell.2009.02.009.
- [6] de la Mata M, Lafaille C, Kornblihtt AR. First come, first served revisited: factors affecting the same alternative splicing event have different effects on the relative rates of intron removal. *RNA.* 2010;5:904–912. Doi: 10.1261/rna.1993510.
- [7] Sims RJ, 3rd, Millhouse S, Chen CF, Lewis BA, Erdjument-Bromage H, Tempst P, et al. Recognition of trimethylated histone H3 lysine 4 facilitates the recruitment of transcription postinitiation factors and pre-mRNA splicing. *Mol Cell.* 2007;4:665–676. Doi: 10.1016/j.molcel.2007.11.010.

- [8] Mauger DM, Lin C, Garcia-Blanco MA. hnRNP H and hnRNP F complex with Fox2 to silence fibroblast growth factor receptor 2 exon IIIc. *Mol Cell Biol.* 2008;17:5403–5419. Doi: 10.1128/MCB.00739-08.
- [9] Venables JP, Lapasset L, Gadea G, Fort P, Klinck R, Irimia M, et al. MBNL1 and RBFOX2 cooperate to establish a splicing programme involved in pluripotent stem cell differentiation. *Nat Commun.* 2013;248010.1038/ncomms3480.
- [10] Mallinjoud P, Villemain JP, Mortada H, Polay Espinoza M, Desmet FO, Samaan S, et al. Endothelial, epithelial, and fibroblast cells exhibit specific splicing programs independently of their tissue of origin. *Genome Res.* 2014;3:511–521. Doi: 10.1101/gr.162933.113.
- [11] Venables JP, Brosseau JP, Gadea G, Klinck R, Prinos P, Beaulieu JF, et al. RBFOX2 is an important regulator of mesenchymal tissue-specific splicing in both normal and cancer tissues. *Mol Cell Biol.* 2013;2:396–405. Doi: 10.1128/MCB.01174-12.
- [12] Danan-Gotthold M, Golan-Gerstl R, Eisenberg E, Meir K, Karni R, Levanon EY. Identification of recurrent regulated alternative splicing events across human solid tumors. *Nucleic Acids Res.* 2015;10:5130–5144. Doi: 10.1093/nar/gkv210.
- [13] Chou YS, Yang MH. Epithelial-mesenchymal transition-related factors in solid tumor and hematological malignancy. *J Chin Med Assoc.* 2015;8:438–445. Doi: 10.1016/j.jcma.2015.05.002.
- [14] Cichon MA, Nelson CM, Radisky DC. Regulation of epithelial-mesenchymal transition in breast cancer cells by cell contact and adhesion. *Cancer informatics.* 2015;Suppl 3:1–13. Doi: 10.4137/CIN.S18965.
- [15] Braeutigam C, Rago L, Rolke A, Waldmeier L, Christofori G, Winter J. The RNA-binding protein Rbfox2: an essential regulator of EMT-driven alternative splicing and a mediator of cellular invasion. *Oncogene.* 2014;9:1082–1092. Doi: 10.1038/onc.2013.50.
- [16] Germann S, Gratadou L, Dutertre M, Auboeuf D. Splicing programs and cancer. *J Nucleic Acids.* 2012; 2012, Article ID: 269570. Doi: 10.1155/2012/269570.
- [17] Shapiro IM, Cheng AW, Flytzanis NC, Balsamo M, Condeelis JS, Oktay MH, et al. An EMT-driven alternative splicing program occurs in human breast cancer and modulates cellular phenotype. *PLoS Genet.* 2011;8:e1002218. Doi: 10.1371/journal.pgen.1002218.
- [18] Venables JP. Aberrant and alternative splicing in cancer. *Cancer Res.* 2004;21:7647–7654. Doi: 10.1158/0008-5472.CAN-04-1910.
- [19] Fackenthal JD, Godley LA. Aberrant RNA splicing and its functional consequences in cancer cells. *Dis Model Mech.* 2008;1:37–42. Doi: 10.1242/dmm.000331.
- [20] Silipo M, Gautrey H, Tyson-Capper A. Deregulation of splicing factors and breast cancer development. *J Mol Cell Biol.* 2015; 7: 388–401. Doi: 10.1093/jmcb/mjv027.
- [21] Patry C, Bouchard L, Labrecque P, Gendron D, Lemieux B, Toutant J, et al. Small interfering RNA-mediated reduction in heterogeneous nuclear ribonucleoparticle A1/

A2 proteins induces apoptosis in human cancer cells but not in normal mortal cell lines. *Cancer Res.* 2003;22:7679–7688.

- [22] He Y, Brown MA, Rothnagel JA, Saunders NA, Smith R. Roles of heterogeneous nuclear ribonucleoproteins A and B in cell proliferation. *J Cell Sci.* 2005;Pt 14:3173–3183. Doi: 10.1242/jcs.02448.
- [23] David CJ, Manley JL. Alternative pre-mRNA splicing regulation in cancer: pathways and programs unhinged. *Genes Dev.* 2010;21:2343–2364. Doi: 10.1101/gad.1973010.
- [24] Li S, Xu H, Ding H, Huang Y, Cao X, Yang G, et al. Identification of an aptamer targeting hnRNP A1 by tissue slide-based SELEX. *J Pathol.* 2009;3:327–336. Doi: 10.1002/path.2543.
- [25] Karni R, de Stanchina E, Lowe SW, Sinha R, Mu D, Krainer AR. The gene encoding the splicing factor SF2/ASF is a proto-oncogene. *Nat Struct Mol Biol.* 2007;3:185–193. Doi: 10.1038/nsmb1209.
- [26] Zhou ZJ, Dai Z, Zhou SL, Fu XT, Zhao YM, Shi YH, et al. Overexpression of HnRNP A1 promotes tumor invasion through regulating CD44v6 and indicates poor prognosis for hepatocellular carcinoma. *Int J Cancer.* 2013;5:1080–1089. Doi: 10.1002/ijc.27742.
- [27] Honore B, Baandrup U, Vorum H. Heterogeneous nuclear ribonucleoproteins F and H/H' show differential expression in normal and selected cancer tissues. *Experiment Cell Res.* 2004;1:199–209. Doi: 10.1016/j.yexcr.2003.11.011.
- [28] Rauch J, Ahlemann M, Schaffrik M, Mack B, Ertongur S, Andratschke M, et al. Allogenic antibody-mediated identification of head and neck cancer antigens. *Biochem Biophys Res Commun.* 2004;1:156–162. Doi: 10.1016/j.bbrc.2004.08.071.
- [29] Rauch J, O'Neill E, Mack B, Matthias C, Munz M, Kolch W, et al. Heterogeneous nuclear ribonucleoprotein H blocks MST2-mediated apoptosis in cancer cells by regulating A-Raf transcription. *Cancer Res.* 2010;4:16791688. Doi: 10.1158/0008-5472.CAN-09-2740.
- [30] Anczukow O, Rosenberg AZ, Akerman M, Das S, Zhan L, Karni R, et al. The splicing factor SRSF1 regulates apoptosis and proliferation to promote mammary epithelial cell transformation. *Nat Struct Mol Biol.* 2012;2:220–228. Doi: 10.1038/nsmb.2207.
- [31] Ghigna C, Giordano S, Shen H, Benvenuto F, Castiglioni F, Comoglio PM, et al. Cell motility is controlled by SF2/ASF through alternative splicing of the Ron protooncogene. *Mol Cell.* 2005;6:881–890. Doi: 10.1016/j.molcel.2005.10.026.
- [32] Gokmen-Polar Y, Neelamraju Y, Goswami CP, Gu X, Nallamotheu G, Janga SC, et al. Expression levels of SF3B3 correlate with prognosis and endocrine resistance in estrogen receptor-positive breast cancer. *Mod Pathol.* 2014;28:677–685. Doi: 10.1038/modpathol.2014.146.

- [33] Hsu TY, Simon LM, Neill NJ, Marcotte R, Sayad A, Bland CS, et al. The spliceosome is a therapeutic vulnerability in MYC-driven cancer. *Nature*. 2015;7569:384–388. Doi: 10.1038/nature14985.
- [34] Ishii H, Saitoh M, Sakamoto K, Kondo T, Katoh R, Tanaka S, et al. Epithelial splicing regulatory proteins 1 (ESRP1) and 2 (ESRP2) suppress cancer cell motility via different mechanisms. *J Biol Chem*. 2014;40:27386–27399. Doi: 10.1074/jbc.M114.589432.
- [35] Lapuk A, Marr H, Jakkula L, Pedro H, Bhattacharya S, Purdom E, et al. Exon-level microarray analyses identify alternative splicing programs in breast cancer. *Mol Cancer Res*. 2010;7:961–974. Doi: 10.1158/1541-7786.MCR-09-0528.
- [36] Ueda J, Matsuda Y, Yamahatsu K, Uchida E, Naito Z, Korc M, et al. Epithelial splicing regulatory protein 1 is a favorable prognostic factor in pancreatic cancer that attenuates pancreatic metastases. *Oncogene*. 2014;36:4485–4495. Doi: 10.1038/onc.2013.392.
- [37] Zhao Q, Caballero OL, Davis ID, Jonasch E, Tamboli P, Yung WK, et al. Tumor-specific isoform switch of the fibroblast growth factor receptor 2 underlies the mesenchymal and malignant phenotypes of clear cell renal cell carcinomas. *Clin Cancer Res*. 2013;9:2460–2472. Doi: 10.1158/1078-0432.CCR-12-3708.
- [38] Zheng X, Carstens JL, Kim J, Scheible M, Kaye J, Sugimoto H, et al. Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. *Nature*. 2015;7579:525–530. Doi: 10.1038/nature16064.
- [39] Lo CW, Kaida D, Nishimura S, Matsuyama A, Yashiroda Y, Taoka H, et al. Inhibition of splicing and nuclear retention of pre-mRNA by spliceostatin A in fission yeast. *Biochem Biophys Res Commun*. 2007;3:573–577. Doi: 10.1016/j.bbrc.2007.10.029.
- [40] Hahn CN, Venugopal P, Scott HS, Hiwase DK. Splice factor mutations and alternative splicing as drivers of hematopoietic malignancy. *Immunol Rev*. 2015;1:257–278. Doi: 10.1111/imr.12241.
- [41] Scott LM, Rebel VI. Acquired mutations that affect pre-mRNA splicing in hematologic malignancies and solid tumors. *J Natl Cancer Inst*. 2013;20:1540–1549. Doi: 10.1093/jnci/djt257.
- [42] Maguire SL, Leonidou A, Wai P, Marchio C, Ng CK, Sapino A, et al. SF3B1 mutations constitute a novel therapeutic target in breast cancer. *J Pathol*. 2014;235:571–580. Doi: 10.1002/path.4483.
- [43] Li X, Manley JL. Inactivation of the SR protein splicing factor ASF/SF2 results in genomic instability. *Cell*. 2005;3:365–378. Doi: 10.1016/j.cell.2005.06.008.
- [44] Ngo JC, Giang K, Chakrabarti S, Ma CT, Huynh N, Hagopian JC, et al. A sliding docking interaction is essential for sequential and processive phosphorylation of an SR protein by SRPK1. *Mol Cell*. 2008;5:563–576. Doi: 10.1016/j.molcel.2007.12.017.
- [45] Gout S, Brambilla E, Boudria A, Drissi R, Lantuejoul S, Gazzeri S, et al. Abnormal expression of the pre-mRNA splicing regulators SRSF1, SRSF2, SRPK1 and SRPK2 in

- non small cell lung carcinoma. PLoS One. 2012;10:e46539. Doi: 10.1371/journal.pone.0046539.
- [46] Ben-Hur V, Denichenko P, Siegfried Z, Maimon A, Krainer A, Davidson B, et al. S6K1 alternative splicing modulates its oncogenic activity and regulates mTORC1. *Cell Reports*. 2013;1:103–115. Doi: 10.1016/j.celrep.2012.11.020.
 - [47] Valacca C, Bonomi S, Buratti E, Pedrotti S, Baralle FE, Sette C, et al. Sam68 regulates EMT through alternative splicing-activated nonsense-mediated mRNA decay of the SF2/ASF proto-oncogene. *J Cell Biol*. 2010;1:87–99. Doi: 10.1083/jcb.201001073.
 - [48] Yadav AK, Vashishta V, Joshi N, Taneja P. AR-A 014418 Used against GSK3beta Downregulates expression of hnRNP A1 and SF2/ASF splicing factors. *J Oncol*. 2014; 2014:9 Article ID 695325. Doi: 10.1155/2014/695325.
 - [49] Mavrou A, Brakspear K, Hamdollah-Zadeh M, Damodaran G, Babaei-Jadidi R, Oxley J, et al. Serine-arginine protein kinase 1 (SRPK1) inhibition as a potential novel targeted therapeutic strategy in prostate cancer. *Oncogene*. 2015;33:4311–4319. Doi: 10.1038/onc.2014.360.
 - [50] Warzecha CC, Shen S, Xing Y, Carstens RP. The epithelial splicing factors ESRP1 and ESRP2 positively and negatively regulate diverse types of alternative splicing events. *RNA Biol*. 2009;5:546–562.
 - [51] Bebee TW, Park JW, Sheridan KI, Warzecha CC, Cieply BW, Rohacek AM, et al. The splicing regulators ESRP1 and ESRP2 direct an epithelial splicing program essential for mammalian development. *eLife*. 2015; 4: 10.7554/eLife.08954.
 - [52] Brown RL, Reinke LM, Damerow MS, Perez D, Chodosh LA, Yang J, et al. CD44 splice isoform switching in human and mouse epithelium is essential for epithelial-mesenchymal transition and breast cancer progression. *J Clin Invest*. 2011;3:1064–1074. Doi: 10.1172/JCI44540.
 - [53] Warzecha CC, Jiang P, Amirikian K, Dittmar KA, Lu H, Shen S, et al. An ESRP-regulated splicing programme is abrogated during the epithelial-mesenchymal transition. *EMBO J*. 2010;19:3286–3300. Doi: 10.1038/emboj.2010.195.
 - [54] Reinke LM, Xu Y, Cheng C. Snail represses the splicing regulator epithelial splicing regulatory protein 1 to promote epithelial-mesenchymal transition. *J Biol Chem*. 2012;43:36435–36442. Doi: 10.1074/jbc.M112.397125.
 - [55] Goel HL, Gritsko T, Pursell B, Chang C, Shultz LD, Greiner DL, et al. Regulated splicing of the alpha6 integrin cytoplasmic domain determines the fate of breast cancer stem cells. *Cell reports*. 2014;3:747–761. Doi: 10.1016/j.celrep.2014.03.059.
 - [56] Di Modugno F, Iapicca P, Boudreau A, Mottolese M, Terrenato I, Perracchio L, et al. Splicing program of human MENA produces a previously undescribed isoform associated with invasive, mesenchymal-like breast tumors. *Proc Natl Acad Sci U S A*. 2012;47:19280–19285. Doi: 10.1073/pnas.1214394109.

- [57] Ranieri D, Belleudi F, Magenta A, Torrisi MR. HPV16 E5 expression induces switching from FGFR2b to FGFR2c and epithelial-mesenchymal transition. *Int J Cancer*. 2015;1:61–72. Doi: 10.1002/ijc.29373.
- [58] Leontieva OV, Ionov Y. RNA-binding motif protein 35A is a novel tumor suppressor for colorectal cancer. *Cell cycle*. 2009;3:490–497.
- [59] Horiguchi K, Sakamoto K, Koinuma D, Semba K, Inoue A, Inoue S, et al. TGF-beta drives epithelial-mesenchymal transition through deltaEF1-mediated downregulation of ESRP. *Oncogene*. 2012;26:3190–3201. Doi: 10.1038/onc.2011.493.
- [60] Roca H, Hernandez J, Weidner S, McEachin RC, Fuller D, Sud S, et al. Transcription factors OVOL1 and OVOL2 induce the mesenchymal to epithelial transition in human cancer. *PLoS One*. 2013;10:e76773. Doi: 10.1371/journal.pone.0076773.
- [61] Yae T, Tsuchihashi K, Ishimoto T, Motohara T, Yoshikawa M, Yoshida GJ, et al. Alternative splicing of CD44 mRNA by ESRP1 enhances lung colonization of metastatic cancer cell. *Nat Commun*. 2012;3:883. Doi: 10.1038/ncomms1892.
- [62] Damianov A, Black DL. Autoregulation of Fox protein expression to produce dominant negative splicing factors. *RNA*. 2010;2:405–416. Doi: 10.1261/rna.1838210.
- [63] Norris JD, Fan D, Sherk A, McDonnell DP. A negative coregulator for the human ER. *Mol Endocrinol*. 2002;3:459–468. Doi: 10.1210/mend.16.3.0787.
- [64] Venables JP, Klinck R, Koh C, Gervais-Bird J, Bramard A, Inkel L, et al. Cancer-associated regulation of alternative splicing. *Nat Struct Mol Biol*. 2009;6:670–676. Doi: 10.1038/nsmb.1608.
- [65] Wen J, Toomer KH, Chen Z, Cai X. Genome-wide analysis of alternative transcripts in human breast cancer. *Breast Cancer Res Treat*. 2015;2:295–307. Doi: 10.1007/s10549-015-3395-2.
- [66] Venables JP, Klinck R, Bramard A, Inkel L, Dufresne-Martin G, Koh C, et al. Identification of alternative splicing markers for breast cancer. *Cancer Res*. 2008;22:9525–9531. Doi: 10.1158/0008-5472.CAN-08-1769.
- [67] Cen YL, Qi ML, Li HG, Su Y, Chen LJ, Lin Y, et al. Associations of polymorphisms in the genes of FGFR2, FGF1, and RBF2 with breast cancer risk by estrogen/progesterone receptor status. *Mol Carcinog*. 2013, 52:E52–59. Doi: 10.1002/mc.21979.