



Alternative splicing is an important mechanism behind KAI1 loss of function in breast cancer patients from Saudi Arabia

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Abstract

Purpose KAI1 (also called CD82) is a metastasis suppressor gene known to be downregulated in breast cancer and other solid tumors. The downregulation of KAI1 or loss of its function is usually associated with bad prognosis. The mechanism behind KAI1 loss of function is complex. In this study, we investigated “alternative splicing” as a possible mechanism that underlies KAI1 loss of function in breast cancer patients from a tertiary hospital in Saudi Arabia.

Methods Expression of KAI1 was studied in FFPE breast cancer and control tissue sections by IHC using two different antibodies targeting different domains of the protein. The TS82B antibody targets the extracellular loop, which constitutes most of the protein, while the second EPR4112 antibody targets the C-terminal intracellular domain of the protein.

Results Out of 90 breast cancer samples, 67% showed loss of KAI1 expression. The remaining 33% showed KAI1 expression with (TS82B) antibody; however, the protein was detected in only 11% of cancers when using the antibody (EPR4112) indicating a truncation of the protein at the C-terminus (truncated-KAI1) in 22% of the studied cancer samples. A significant correlation was found between truncated-KAI1 expression and advanced cancer stage (association with lymph node metastasis, *P* value 0.008).

Conclusion Alternative splicing is an important mechanism underlying KAI1 loss of function in breast cancer, and it is associated with bad prognosis (advanced cancer stage).

Keywords KAI1 downregulation · Metastasis suppressor genes · Breast cancer · Alternative splicing · Splice variant · KAI1 expression by IHC

Background

Breast cancer high mortality rate depends mainly on its progression through invasion and metastases. Cancer spread passes through several steps called “metastatic cascade” which includes cancer cell migration, vascular invasion, extravasations, and finally survival and proliferation at a distant site [1]. Several metastases promoting or suppressing genes have been identified, which encode oncoproteins or metastasis suppressor proteins, respectively. Metastasis suppressors are proteins inhibiting the metastasis process usually with no effect on the initial cancer growth [2–9]. Among metastasis suppressors is the Kangai1 protein (KAI1, also called CD82), a transmembrane glycoprotein known to be downregulated in different types of cancers with high metastatic potential [2–9]. KAI1 is involved in several metastasis processes starting from fusion, adhesion, and migration to apoptosis and cell shape modifications. Therefore, it inhibits

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cancer metastasis by suppressing cancer cell movement and invasiveness [1, 6, 8, 10]. In cell membrane, KAI1 is juxtaposed with proteins essential for cell transmigration such as integrin $\alpha 1\beta 3$ and E-cadherin [11]. KAI1 interacts with these proteins to prevent cell migration [12]. KAI1 also organizes the trafficking of proteins through cell membrane which has an effect on several signaling pathways [13, 14]. Complex mechanisms may lead to KAI1 loss of expression, which includes regulating transcription, alternative splicing [15, 16] and even posttranslational remodeling [17–19]. There is limited evidence for gene mutation, loss of heterozygosity (LOH) [20], promoter mutation or hypermethylation [21, 22] as a cause of KAI1 downregulation. For therapeutic targeting of KAI1, it is crucial to understand the mechanisms affecting its expression and regulation during cancer development [18, 23–25].

Alternative splicing is a known mechanism behind KAI1 loss of function. Lee et al. described an alternately spliced KAI1 lacking 28 amino acids from its distal part (due to deletion of exon7) [16, 26]. This truncation of the C-terminal region results in activation of VANGL1 (KITENIN), a metastases enhancer which usually loses its effects when interacting with KAI1 c-terminal cytoplasmic domain indicating the crucial role of the C-terminus in metastases suppressor function of KAI1. Moreover, Upheber et al. reported in 2015 that deletion of exon7 from KAI1 leads to a non-functional protein losing its metastasis suppressive effect [27]. Moreover, the truncated-KAI1 also promotes cancer cell proliferation [27]. Other than exon7 deletion, a less known splice-KAI1 variant has been reported by Moez et al. in 2011 which includes a 274-bp intronic insertion between exon 8 & 9 making the RNA unstable and leading to structural changes affecting its function [5].

KAI1 expression and/or alternative splicing was not studied previously from patients in Saudi Arabia. We aimed here to evaluate KAI1 expression by IHC and elucidate the possibility of alternative splicing as a mechanism of KAI1 loss of function in breast cancer patients from Saudi Arabia.

Methods

Sample collection

From the archive (2009–2014) of the Pathology Department at King Fahd Hospital of the University, we collected 90 formalin-fixed paraffin-embedded (FFPE) tissue specimens from breast cancer samples along with 38 metastatic lymph node samples and 70 FFPE normal breast tissue samples (controls) from the same patients whenever available (safety margins). All samples were from mastectomy or lumpectomy. No core biopsy was included because of the limited cancer representation in such samples. All hematoxylin and

eosin (H&E) slides from cancer, lymph node, and control samples have been reexamined by two pathologists who confirmed the diagnosis and the quality of each specimen.

The patients' age at diagnosis ranges from 21 to 75 years (median age was 48 years). The majority of breast cancer cases (90%) were invasive ductal, NOS (not otherwise specified). Eight cases were of lobular type, one was mucinous, and 4 cases were in-situ ductal carcinoma. The majority (90%) of cases were grade II or III, while only 10% were grade I. Almost, 50% of cancer cases were associated with histologically proven metastatic lymph nodes.

This project was reviewed and approved at the Imam Abdulrahman Bin Faisal University IRB institutional review board (ethical committee) meeting on Sunday, April 26, 2015. IRB number: IRB-2015-01-095. Informed patient consent was waived due to use of archival material of anonymous nature that does not disclose patients identity and because patients are lost to follow up.

Immunohistochemical study (IHC)

Using Ventana Automated IHC stainer along with Ventana Ultraview universal DAB detection kit, we performed IHC procedures on all tissue specimens (3–4- μ m-thick paraffin sections) following the standard protocol according to the manufacturer's instructions. Sections were counter-stained by hematoxylin. The primary antibodies were used at the recommended dilution (1/200) and incubation time 32 min. Two different anti-CD82 primary antibodies (different clones targeting different epitopes) were used. The first anti-CD82 (TS82B, Abcam, Cambridge, UK) was applied to all samples (90 cancer tissues, 38 metastatic lymph nodes, and 70 control tissues). This TS82B clone recognizes the antigen somewhere at the large extracellular loop comprising amino acids 17–242, excluding the cytoplasmic loop of the protein (aa73–83) (Katarzyna Dudek, Abcam plc, personal communication) (Fig. 1). On the second step, a different anti-CD82 antibody (EPR4112, Abcam, Cambridge, UK) was applied on the cancer samples that were positive in the first run (with the TS82B antibody) along with 22 non-cancer control samples. The anti-CD82 EPR4112 clone is a monoclonal antibody, targeting the intracellular domain on the C-terminal end after the last helical transmembrane domain (Ralph Paul, Abcam Inc. personal communication) (Fig. 1). The IHC expression was analyzed by two pathologists and classified into two categories: negative or positive (partial or diffuse expression).

Data analysis

Data analysis was performed using the IBM SPSS software v. 24. We utilized mainly two functions: frequencies and correlation (including Pearson coefficient and test of

Fig. 1 Domains in KAI1 (wild-type and splice variant) targeted by the primary anti-KAI1 antibodies TS82B & EPR4112

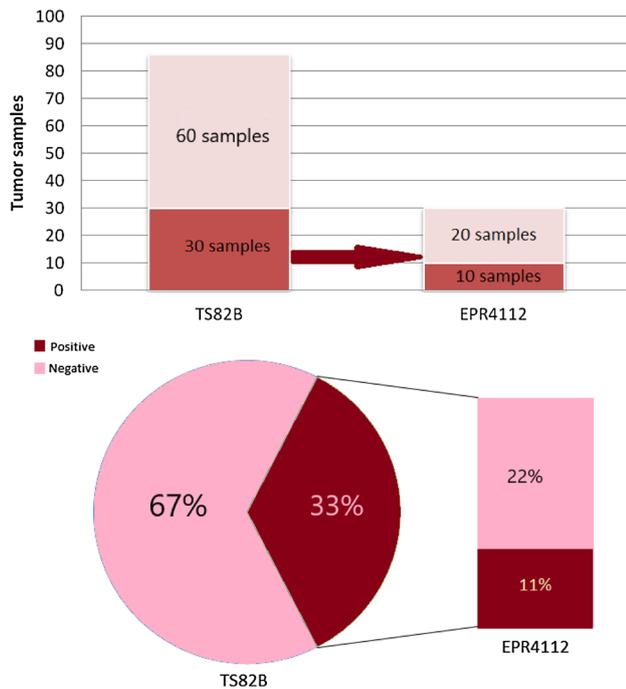
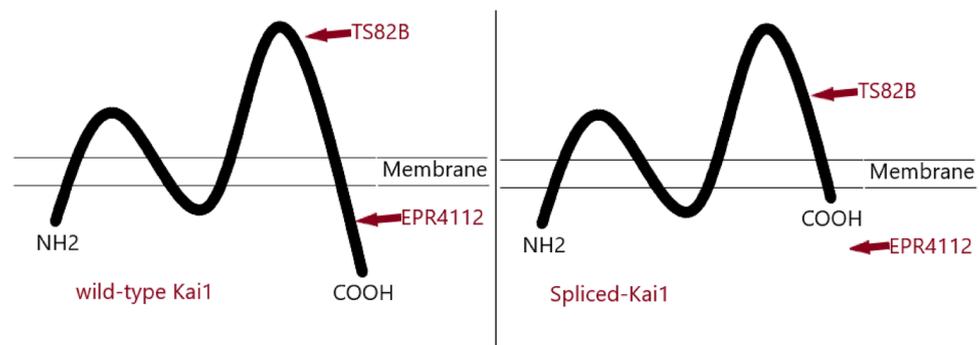


Fig. 2 Schematic illustration of KAI1 expression in cancer samples

significance two-tailed). Significance association was considered whenever P value is less than 0.05.

Results

KAI1 expression profile by IHC

In the first step, a total of 90 cancer samples were tested for the expression of KAI1 by IHC using anti-CD82 (TS82B). While 60 cancer samples showed complete loss of KAI1 expression, only 30 cancer samples were positive (expressing KAI1 protein) (Fig. 2). In a second step, these 30 cancer samples, expressing KAI1 in the first step, have been re-analyzed by another anti-CD82 (EPR4112) which only detects the preserved c-terminal region of wild-type KAI1 (Fig. 3c). Only 10/30 cancer samples were positive (so expressing

wild-type KAI1). The remaining 20/30 cancer samples were negative by EPR4112 antibody in cancer cells (so expressing the truncated-KAI1) while positive in the adjacent lymphocytes (expressing the wild-type KAI1) (Fig. 3d). Furthermore, All 70 control samples tested with TS82B antibody were positive (Fig. 3a) and all the 22 control samples tested with EPR4112 antibody were positive (Fig. 3b).

A similar IHC method was applied on 38 metastatic lymph node samples and showed comparable results with its counterpart in the primary site. No significant difference in KAI1 expression was noticed in cancer cases between the primary site and the metastatic site.

Data analysis: correlation of KAI1 expression to different cancer parameters

Statistical analysis using SPSS v.24 showed a significant correlation of KAI1 loss of expression to ER/PR loss of expression and Her2 over-expression (P value 0.000, 0.05, 0.048, respectively). No significant correlation was found with cancer grade, cancer type, age of patient, or P53 expression. On the other hand, there was a significant correlation between cancer expressing the truncated-KAI1 and the lymph node metastasis (P value 0.008).

Discussion

Similar to other types of cancer, KAI1 expression is down-regulated in breast cancer, especially in the advanced stages. However, our findings revealed a preserved KAI1 expression in almost one-third of the studied cancer samples. These cases were mostly of advanced stages and associated with lymph node metastasis. To explain this discordance, we supposed that the expressed KAI1 was non-functional. One of the mechanisms behind KAI1 loss of function is gene alternative splicing. Deletion of exon7 is the most commonly known alternative splicing of KAI1 which results in a protein lacking 28 amino acids from its distal part leading to a structural alteration in the intracellular domain within the c-terminal region of the protein [16]. This alteration makes

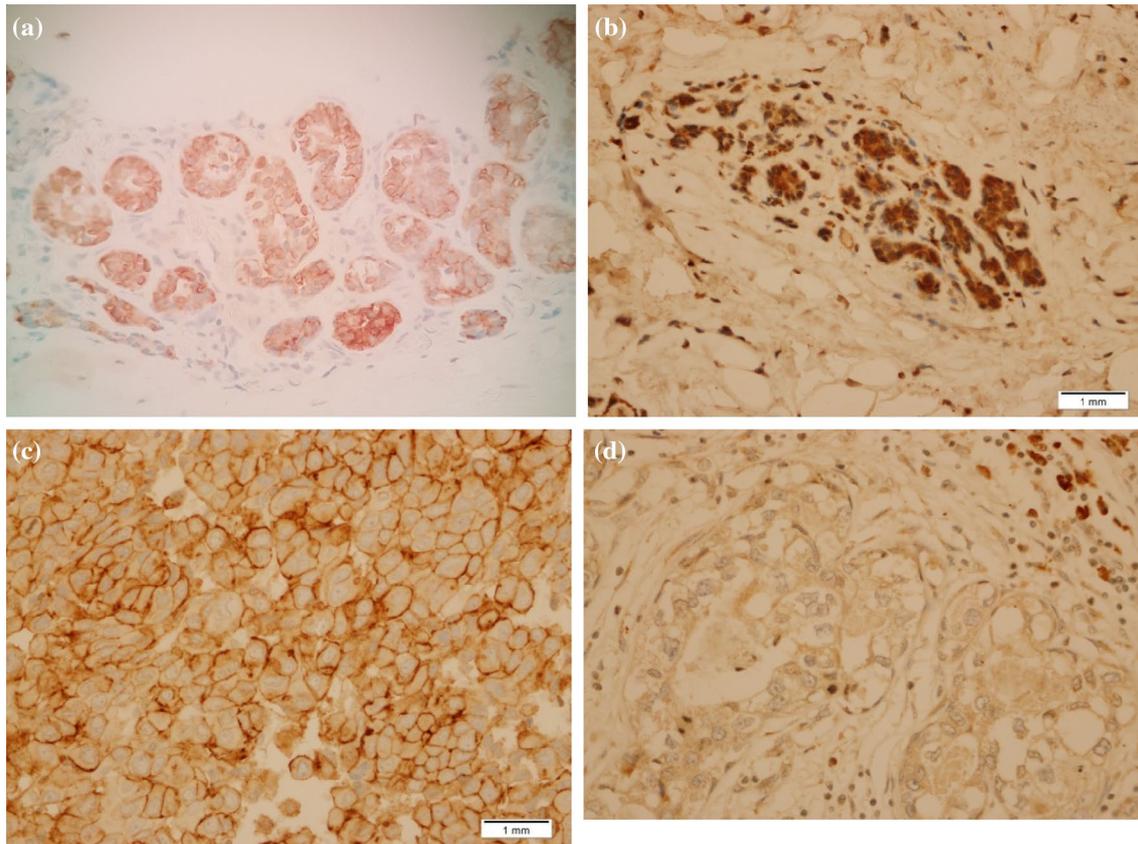


Fig. 3 **a** Microscopic image $\times 20$ shows (TS82B) positive in breast normal tissue. **b** Microscopic image $\times 20$ shows (EPR4112) positive in breast normal tissue. **c** Microscopic image $\times 20$ shows (TS82B)

positive in breast cancer tissue. **d** Microscopic image $\times 20$ shows (EPR4112) negative in breast cancer tissue while positive in lymphocytes

KAI1 undetectable by an anti-CD82 targeting a narrow epitope within the c-terminal region (such as EPR4112), while it is detectable by anti-CD82 targeting a different epitope at the large extracellular loop (such as TS82B) (Fig. 3).

We found that two-thirds of the cancer samples positive for anti-CD82 (TS82B) were negative when using anti-CD82 (EPR4112), indicating the presence of an altered c-terminal intracellular domain (truncated-KAI1) in those cancers; however, the protein was preserved in the adjacent normal lymphocytes which were positive for EPR4112 (lymphocytes considered as an internal control for the antibody).

It is known that the KAI1 c-terminal region has a crucial role in KAI1 function [26], in addition to the loss of metastasis suppression; the alteration of c-terminal region promotes cancer proliferation [27].

Our findings showed a significant correlation between cancer cases expressing the truncated-KAI1 and the presence of lymph node metastasis. In other words, the majority of cancer samples probably expressing wt-KAI1 (positive for both TS82B & EPR4112) were of early stage (free lymph nodes), while most of the cancer samples

expressing truncated-KAI1 (positive only for TS82B) were associated with lymph node metastasis.

IHC methodology is simple, cost-effective, and reliable in detecting truncated-KAI1 in which c-terminal region is altered (exon7 deletion); however, it is not capable of detecting another less known splice-variants [5].

Conclusion

Alternative splicing is an important mechanism underlying KAI1 loss of function in breast cancers from Saudi Arabia, and it is significantly associated with advanced cancer stage.

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Data availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests in the manuscript.

Ethics approval This project was reviewed and approved at the Imam Abdulrahman Bin Faisal University IRB institutional review board (ethical committee) meeting on Sunday, April 26, 2015. IRB Number: IRB-2015-01-095.

Informed consent Informed patient consent was waived due to use of archival material of anonymous nature that does not disclose patients identity and because patients are lost to follow up.

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