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Significance of Immunohistochemical Expression of Fascin-1 in Colorectal Carcinoma

Maisa Hashem Mohammed*, Emad Ali Ahmed, Tasneem Mohammed Bakheet***, Nagwa Abd El-Sadek Ahmed***

* Department of Pathology, ** Department of General Surgery,
*** Department of Public Health and Community Medicine, Faculty of Medicine, Sohag University, Egypt
Corresponding author: Maisa Hashem Mohammed, **E-mail**: maisaahashem@med.sohag.edu.eg,
Tel. 01060260461, Orcid Id: 0000-0002-0939-7097

ABSTRACT

Introduction: Colorectal carcinoma (CRC) is the third most prevalent cancer and the second leading cause of cancer-related mortality. The high morbidity and mortality rates associated with CRC are attributed to the ability of the neoplastic cells to metastasize to distant sites. Fascin actin-bundling protein-1 (FSCN-1) is a member of Fascin family. Its expression is up regulated in various types of neoplasms. Previous studies reported that Fascin-1 is responsible for enhancing both invasive and metastatic potentials of neoplastic cells by modulating cellular and extracellular properties. Fortunately, FSCN-1 can be blocked by new therapeutic agents.

Objectives: This study aimed to evaluate expression of FSCN-1 in colorectal carcinoma (CRC) and to correlate its expression with the available clinicopathological parameters to assess its prognostic value.

Methods: Paired formalin-fixed, paraffin embedded tissue blocks of 60 cases of CRC and their adjacent normal colonic mucosa were included in this study. FSCN-1 expression was evaluated by immunohistochemistry (IHC). Correlation of different levels of Fascin-1 expression with different clinicopathological parameters were statistically analyzed.

Results: All cases of CRC showed immunohistochemical expression of FSCN-1 with variable staining intensities and extents. FSCN-1 expression showed positive statistically significant correlations with tumor grade ($p = 0.002$), pathological T stage ($p< 0.005$), nodal metastasis ($p<0.006$), and vascular invasion ($p = 0.001$).

Conclusion: FSCN-1 is an independent adverse prognostic factor in CRC. Its overexpression could be used as an indicator of tumor progression and metastasis. It could be targeted in future therapeutic approaches to decrease CRC progression and spread.

Keywords: Fascin-1, Colorectal carcinoma, Metastasis, Cytoskeleton, Vascular invasion.

INTRODUCTION

Colorectal carcinoma (CRC) is the third most prevalent globally distributed malignant neoplasm. It represents the second leading cause of cancer-related mortality. CRC is a major public health problem. In Egypt, it is the 7th most prevalent cancer in the Egyptian population [(1)]. The high morbidity and mortality rates, which accompany CRC are assigned to the ability of these neoplastic cells to metastasize to distant sites, approximately 50% of CRC are associated with metastasis. Histologically, adenocarcinoma constitutes more than 90% of all CRC cases. Other histopathological variants of CRC include mucinous, Signet ring, medullary, micropapillary, and cribriform phenotypes [(2)].

Metastasis is a multistep process which is initiated at the molecular level in the form of cumulative acquisition of intracellular genetic mutations. These genetic mutations produce a series of dynamic changes in both neoplastic cells and surrounding tissues. Neoplastic cells separate from the primary sites, degrade and invade through basement membranes and extracellular matrix (ECM), invade a vascular channel and/or a serous sac and lastly get arrested in a target organ [(3)]. At these secondary sites, neoplastic cells must adapt themselves in the new microenvironment, evade apoptosis and immunological surveillance, and induce angiogenesis for their survival [(4)]. Initiation of the cell movement is achieved by polymerization of actin into filaments. The actin filaments are packed and bundled at the leading edge of the cell to create membrane protrusions and subsequent different cellular changes as filopodia, lamellipodia and microspikes [(5)].

Detection of genetic aberrations involved in different stages of CRC progression is considered as a major obstacle. Strenuous efforts have been spent to detect metastasis-related biological molecules that can be used either as prognostic markers to predict the risk of tumor progression, or can be blocked to inhibit metastasis [(6)]. Fascin actin-bundling protein 1 (FSCN-1), also known as Fascin-1 or Fascin, is a member of Fascin family. There are three isoforms of Fascin protein family. Fascin-1, is more widely distributed in different human tissues. It stabilizes actin microfilaments into tight and parallel bundles, creating different cellular shapes as microspikes, filopodia and lamellipodia, all of these cellular morphological changes are involved in cell migration, adhesion and cell-cell interactions [(7)].

Physiologically, FSCN-1 is restricted to neural, endothelial, dendritic and mesenchymal cells, while it is absent or minimally expressed in normal epithelial cells [(8)]. In human neoplasms, expression of FSCN-1 is increased, especially in malignant tumors, which show epithelial-mesenchymal transition (EMT). The latter gives the malignant epithelial cells both the migratory and invasive properties [(7, 9)]. FSCN-1 was detected in different human cancers as esophageal squamous cell carcinoma, non-small cell lung cancer, mammary, gastric and pancreatic duct adenocarcinoma [(10-14)]. Fortunately, FSCN-1 can be blocked by new therapeutic

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agents, so FSCN-1 can be used as both a prognostic biomarker and a potential therapeutic target [15].

This study aimed to evaluate the immunohistochemical expression of Fascin-1 in colorectal adenocarcinoma, as it represents the most frequent histological phenotype, and to correlate levels of Fascin-1 expression with clinical parameters as ages and sexes of the included patients, and pathological parameters as tumor differentiation grades, tumor invasion (T category), lymph node status (N category), vascular and perineural invasion.

MATERIALS AND METHODS

Clinical data and specimens' collection

Sixty patients suffered from colorectal carcinoma (CRC) were enrolled in this study. The patients were admitted to Department of General Surgery, Sohag University Hospital from January, 2021 to December of the same year. Patients' complaints were bleeding per rectum, change in bowel habits, abdominal distension, and acute intestinal obstruction, in 39, 14, 5 and 2 patients respectively. Lower endoscopic guided colonic biopsies were done for the patients. The obtained specimens were sent to Pathology laboratory, Sohag Faculty of Medicine, where formalin fixed and paraffin embedded tissue blocks were prepared, then Hematoxylin and Eosin (H & E) tissue sections were examined. H & E-stained slides established the diagnosis of colorectal carcinoma. After that, all the patients underwent colectomy operations and the specimens labeled with patient's name, age, sex and site of the tumor inside the colon were sent to Pathology laboratory. From each specimen; paired formalin fixed and paraffin embedded tissue blocks were obtained from the tumor and the nearby normal colonic mucosa. The studied cases of CRC were staged according to the Tumor-Node-Metastasis [TNM) staging system of the American Joint Committee on Cancer (AJCC), 8th edition] [13].

Inclusion criterion: All cases of CRC obtained by colectomy operations.

Exclusion criteria: Patients who received pre-operative chemotherapy and cases with extensive tumor necrosis.

Ethical considerations: The study was approved by the Committee of Medical Ethics at Sohag Faculty of Medicine, Sohag University (Registration number: ***Soh-Med-22-09-14***). An informed written consent was taken from each participant in this study. The study was registered in Clinical Trials.gov PRS (Clinical Trials.gov ID***: NCT05553080***).

Immunohistochemical staining:

From each paraffin block, 4 µm-thick tissue sections were mounted on positively charged slides for immunostaining (Catalog P/N.0303-7141, China). Paraffin wax was removed from section by using hot and cold xylene, 10 minutes each. Then, sections were placed in a series of descending grades of ethyl alcohol in order to rehydrate them. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 10 minutes. Sections were boiled in Citrate buffer solution (PH 6), for 20 minutes, in order to retrieve antigens and expose cellular epitopes. After being washed by phosphate buffer solution (PBS), tissues were incubated with mouse anti-human Fascin monoclonal primary antibody (Catalog number # MAB7745, mouse IgG2A clone # 833223, Biotechne, at dilution 1:100) overnight. Biotinylated goat polyvalent and Streptavidin peroxidase were applied for 10 minutes each. Diaminobenzidine (DAB) chromogen was used to detect intensity and extent of the primary antibody. Nuclear counterstaining with Mayer's Hematoxylin was used to detect intracellular distribution of Fascin. Universal staining kit (Cat # TP-015-HD, LABVISION Corporation, Fremont, USA) was used. Tissue sections were dehydrated in ascending grades of alcohol, cleared in xylene, and cover slipped.

Positive and negative controls

A human liver tissue was used as a positive control, as recommended by the data sheet, to confirm FSCN-1 immunoreactivity in each series of experiments. FSCN-1 was also expressed in the endothelial cells of blood vessels located in the tumor stroma and was used as an internal control. Sections from human liver tissues were used as a negative control when PBS was used instead of primary anti FSCN-1 antibody.

Immunohistochemical interpretation:

FSCN-1 expression was detected as brownish, granular, cytoplasmic and membranous stain **(Figure 1).** The immunostained sections were scored on the basis of a well-established immunoreactivity scoring system (IRS). The intensity of FSCN-1 immune-expression was scored as follows: 0 (negative staining), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positive tumor cells was scored as follows: 0= (0%), 1 (< 10 %), 2 (10-50%), 3 (51- 80%), and 4 (>80%). The final score was calculated by multiplying the intensity score with the percentage score of positive cells. The resulting IRS was categorized as negative (0), low (1-4), intermediate (6-8) and high staining (9-12) [16].

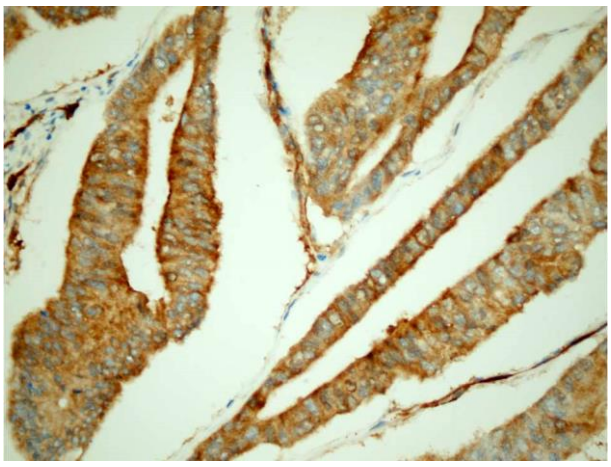

Figure (1): Cytoplasmic and membranous expression of FSCN-1 in colorectal adenocarcinoma, X400.

Statistical analysis

Data were analyzed using SPSS program version 20. Quantitative data were expressed as means ± standard deviation (SD), median and range. Qualitative data were expressed as number and percentage. Chi-square test (χ^2) was used to assess correlations between FSCN-1 expression and the clinicopathological characteristics. P ≤ 0.05 was considered statistically significant.

RESULTS

Patients' characteristics

The current study included 60 patients with CRC. They were 36 men (60%) and 24 women (40%), with male to female ratio was 1.5:1. Patients' ages ranged from 31-76 years, with mean of 54.50 ± 9.10, the median age was 55.50 years. All the enrolled cases were diagnosed as adenocarcinoma of the colon and rectum with variable degrees of differentiation. Well differentiated adenocarcinoma were detected in 36 cases (60%), while the remaining 24 cases (40%) showed moderate degrees of differentiation.

On applying Tumor-Node-Metastasis (TNM) staging system of the American Joint Committee on Cancer (AJCC) 8th edition, 24 (40%) patients were staged as T2, 30 (50%) patients were staged as T3, and T4a was detected in 6 (10%) patients.

Mucoid differentiation was found only in 4 (6.7%) specimens. Involvement of the regional lymph nodes by malignant epithelial cells with glandular morphology was detected in 39 (65%) of the studied patients. Vascular as well as circumferential perineural invasions were detected in 36 (60%) and 10 (16.7%) cases, respectively (Table 1).

Table (1): The clinicopathological characteristics of the studied cases

Clinicopathological parameters	Number of studied patients.	%
Sex		
Male	36	60%
Female	24	40%
Age (years)		
≤50	21	35%
>50	39	65%
Degree of tumor differentiation		
Well differentiation		
Moderate differentiation	36	60%
	24	40%
Mucoid differentiation		
Positive	4	6.7%
Negative	56	93.3%
T category		
T2	24	40%
T3	30	50%
T4a	6	10%
N category		
N0	21	35%
N1	28	46.7%
N2	11	18.3%
Vascular invasion		
Positive	36	60%
Negative	24	40%
Perineural invasion		
Positive	10	16.7%
Negative	50	83.3%

Immunohistochemical expression of FSCN-1:

Immunostaining of FSCN-1 appeared as a brownish cytoplasmic granular stain in the tumor cells and vascular endothelial cells. Normal colonic mucosa adjacent to the tumors were negative for FSCN-1 immunostaining. As regards the examined colorectal adenocarcinoma sections, high levels of FSCN-1 expression was detected in 41 cases, while 14 and 5 cases showed moderate and low levels of FSCN-1 expression, respectively (Figure 2).

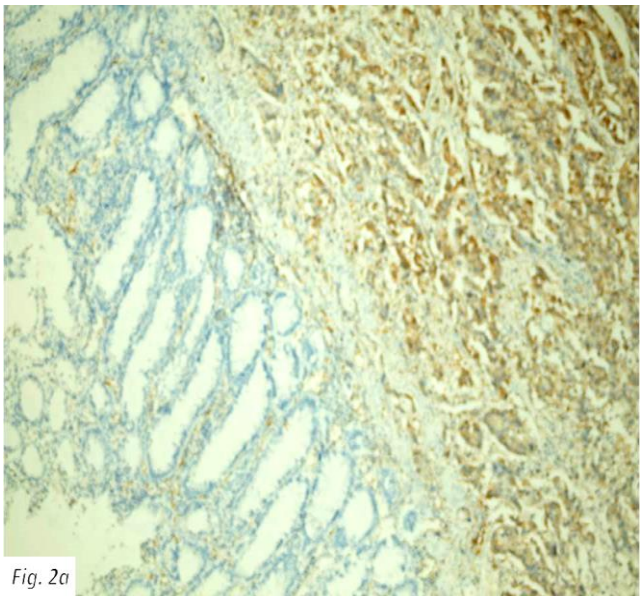

Figure (2a): Cytoplasmic expression of FSCN-1 in neoplastic cells compared to its absence in apparently normal colonic mucosa adjacent to tumor tissue, X: 100.

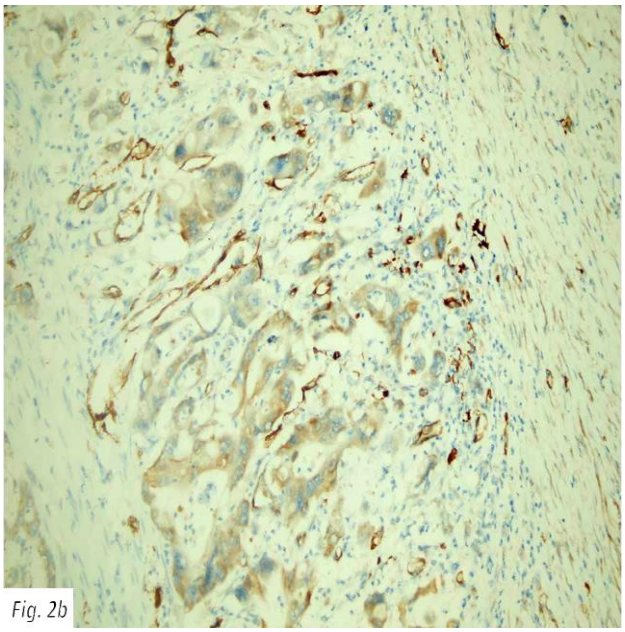

Figure (2b): Expression of FSCN-1 in vascular endothelium was used as internal positive control for FSCN-1 staining, X: 200.

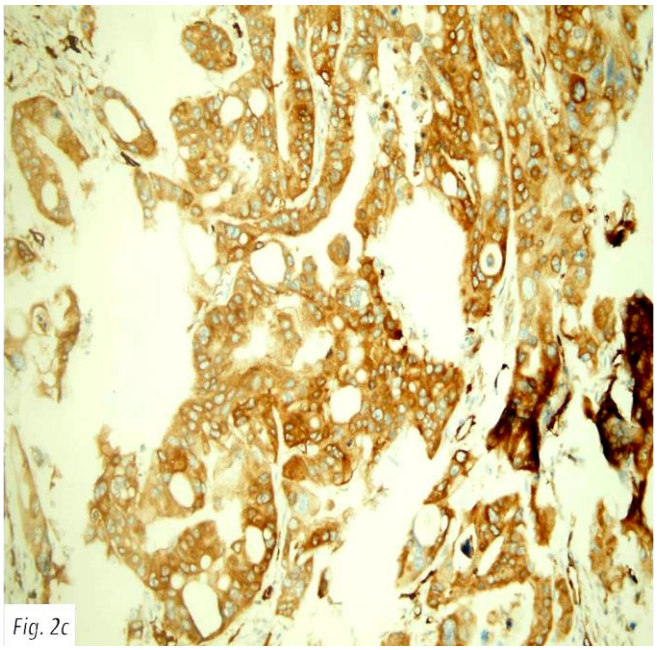

Figure (2c): Strong expression of FSCN-1 in moderately differentiated CRC, X: 200.

A statistically significant relationship was observed between Fascin-1 expression in neoplastic cells and degrees of tumor differentiation. 60% of cases of colorectal carcinoma which showed low levels of Fascin-1 expression and all cases of moderate levels of Fascin-1 expression were well differentiated adenocarcinoma (p= 0.002). Another significant association was found between levels of Fascin-1 expression and the pathological T category of the applied TNM staging system. All cases of pathological T2 category showed low levels of Fascin-1 expression, while all the included cases of T4a category revealed high Fascin-1 expression (P< 0.005).

When levels of Fascin-1 expression were compared to nodal metastasis, all cases of low Fascin-1 expression in addition to 92.86% of cases of moderate Fascin-1 expression were free from neoplastic deposits in their examined lymph nodes. On the other hand, all the examined cases of colorectal carcinoma, which had neoplastic deposits in their lymph nodes, revealed moderate and strong levels of Fascin-1 expression (*p*< 0.006). High levels of Fascin-1 expression were detected in 75.61% of cases of colorectal carcinoma, which had vascular emboli, while 80% of cases which didn't harbor vascular emboli in their examined Hematoxylin and Eosin-stained section showed low levels of Fascin-1 expression (p= 0.001). No significant associations were detected between levels of Fascin-1 expression on one hand and patients' ages, sex, mucoid differentiation and perineural differentiation (Table 2).

Table (2) Clinicopathological characteristics in relation to Fascin-1 expression, n=60

Tested parameter	FSCN-1 Expression						*P* value
	Low levels of expression N=5		Moderate levels of expression N=14		High levels of expression N=41		
	No	%	No	%	No	%	
Sex							
Male	2	40%	11	78.57%	23	56.1%	0.20
female	3	60%	3	21.43%	18	43.9%	
Age (years)							
≤50	3	60%	7	50%	11	26.83%	0.138
>50	2	40%	7	50%	30	73.17%	
Degree of tumor differentiation							
Well differentiated	3	60%	14	100%	19	46.34%	
Moderately differentiated	2	40%	0	0%	22	53.66%	0.002*
Mucoid differentiation							
Present							
Absent	0	0%	0	0%	4	9.76%	0.30
	5	100%	14	100%	37	90.24%	
T category							
T2	5	100%	13	92.86%	6	14.63%	
T3	0	0%	1	7.14%	29	70.73%	< 0.005*
T4a	0	0%	0	0%	6	14.63%	
N category							
N0	5	100%	13	92.86%	3	7.32%	
N1	0	0%	0	0%	28	68.29%	<0.006*
N2	0	0%	1	7.14%	10	24.39%	
Vascular invasion							
Present	1	20%	4	28.57%	31	75.61%	0.001*
Absent	4	80%	10	71.43%	10	24.39%	
Perineural invasion							
Present	0	0%	1	7.14%	9	21.95%	0.25
Absent	5	100%	13	92.86%	32	78.05%	

P value was calculated by Fisher's Exact test, *= significant.

DISCUSSION

Colorectal carcinoma (CRC) is an aggressive malignant epithelial neoplasm of the gastrointestinal tract. It showed cumulative accumulations of genetic aberrations, the latter explain the high morbidity and mortality rates which characterize this disease. Most of the aggressive entities of any malignant neoplasm rely chiefly on the metastatic potential of that neoplasm. CRC is associated with high incidence of metastasis [(17)]. The development of therapeutic agents that specifically inhibit CRC progression and metastasis is a target focus of recent researches [(9)].

FSCN-1 is a member of Fascin family proteins, it is formed of 493 amino acids and weights 55 KDa [(18)]. FSCN-1 polymerizes the intracellular actin filaments in the form of bundles, creating variable morphological features in the cell membranes as microspikes, lamellipodia and filopodia [(8)]. All of these morphological entities are essential for cell migration, cell-cell interactions and cell-matrix adhesions. Physiologically, FSCN-1 is expressed in neuronal, vascular endothelial, mesenchymal and dendritic cells. FSCN-1 is absent or minimally expressed in normal epithelial cells. However, it is re-expressed in different human malignant epithelial neoplasms, which are characterized by epithelial-mesenchymal transition (EMT) in which the neoplastic cells become more aggressive and invasive [(7, 9)].

Increasing evidence points to FSCN-1 protein as a potential prognostic factor in different human malignant neoplasms as its over-expression is detected in variable

malignant tumors as esophageal, lung, mammary, gastric and pancreatic carcinoma, such over-expression was associated with progressive behavior of these neoplasms [10-14].

In the current study, immunohistochemical expression of FSCN-1 was evaluated in 60 CRC specimens and their nearby normal colonic mucosa. All the enrolled cases of CRC expressed FSCN-1 with variable staining intensity and extent, while the adjacent normal mucosa didn't express FSCN-1. This is keeping with what was observed by **Oh *et al*.** [19] where they found that FSCN-1 was expressed only in neoplastic colonic tissues, but not in the normal adjacent colonic mucosa, the investigators also found that FSCN-1 expression was associated with distant metastasis and poor survival. **Wang *et al*.** [20] compared immunohistochemical expression of Resistin and FSCN-1 in 360 cases of CRC in addition to 77 samples of adjacent normal colonic mucosa of Chinese population. They postulated that expression of these two biological markers was accentuated in neoplastic rather than normal colonic mucosa.

We also found that high levels of FSCN-1 expression were significantly associated with increased depth of tumor invasion (T category), presence of nodal metastasis (N category) and higher tumor grades. This is in line with what was observed by **Tsai *et al*.** [21] where they detected the immunohistochemical expression of FSCN-1 in 113 cases of colorectal carcinoma and adenomas with variable dysplastic features. Their study revealed that high levels of FSCN-1 immunostaining were associated with highly dysplastic colorectal adenomas and less differentiated, deeply invasive CRC with advanced nodal stages. We could explain these findings on the basis that normal epithelial cells secrete insoluble extracellular matrix, to which they adhere. Attachment of epithelial cells to their extracellular matrix ensures cellular survival, division and formation of well-organized structures. This is called anchorage dependency. Detachment of normal epithelial cells from their extracellular matrix will result in their apoptosis [22]. Enhanced expression of Fascin family proteins is associated with loss of anchorage dependency [23]. We supposed that over-expression of FSCN-1 in the neoplastic CRC cells has enabled the neoplastic cells to survive and divide without attachment to extracellular matrix. Uncontrolled survival and division will acquire the neoplastic cells additional genetic mutations, which will produce less differentiated, deeply invasive and metastatic tumors.

Additionally, we detected a significant correlation between high levels of FSCN-1 expression and presence of vascular invasion. In a study performed by **Stewart and Crook** [24], they correlated immunohistochemical scores of Fascin expression in undifferentiated and dedifferentiated endometrial carcinoma to lymphovascular invasion. They found that enhanced expression of Fascin was associated with presence of lymphovascular tumor emboli. They postulated that overexpression of Fascin is associated with highly invasive characteristics. Another explanation of this finding is that augmented expression of FSCN-1 results in reconstruction of intracellular actin filaments into well-organized bundles, creating different morphological features as microspikes, lamellipodia and filopodia [8]. All of these morphological entities will augment the invasive potential of the neoplastic cells.

CONCLUSION

Augmented FSCN-1 expression was strongly linked to less differentiated, deeply invasive, nodal and vascular metastasizing CRC. So, it seems to be helpful to target FSCN-1 in future therapeutic approaches for CRC.

Abbreviations: CRC: Colorectal carcinoma, **FSCN-1:** Fascin-1, **EMT:** Epithelial-mesenchymal transition**, H&E:** Hematoxylin and Eosin, **SPSS:** Statistical Package for Social Science.

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