REVIEW ARTICLE

# Advanced Molecular Inhibitory Strategies for Fascin, a Metastatic Biomarker

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**ABSTRACT**
*Cancer is one of the major causes of morbidity and mortality to human population that leads to almost 7.6 million deaths per year globally. Invasion and migration are the main characteristics in the all types of cancers. To combat this deadly disease, prophylaxis, quick diagnosis, researching novel molecular adjuvant therapeutics or the combination of all these strategies are strictly required. Fascin, a 55 kD globular protein is expressed in the metastatic tumors and not in the normal cells. Up-regulation of Fascin has been reported in more aggressive and advanced stage of metastatic cancers. It is an indicator of poor prognosis and late stage of carcinoma. Fascin has two important actin binding sites that help in the invasion and metastasis. New molecular research is focused to inhibit its binding activity by knockdown of Fascin using short hairpin RNA (shRNA), ectopic expression of microRNAs (miRNAs), small interfering RNA (siRNA), using small fascin inhibitors, monoubiquitination and use of antimicrobial peptides. Meta-analysis and systematic review will help a lot to develop new therapeutic strategies that can inhibit tumor cell invasion and even metastatic spread of various carcinomas.*

**KEYWORDS**: *Metastatic carcinoma, Fascin, Invasion, Metastasis, MicroRNAs, Biomarker.*

## INTRODUCTION

Based on the published statistical data by World Health Organization (WHO) globally, the death rates due to cancer is 7.6 million per year. As the world's population is growing day by day, death rate due to cancer is supposed to increase up to 13.1 million/year in the coming couple of decades.[1] Due to these high mortality rates, cancer is considered as a major and the most dangerous cause to the health status of humans and this is the reason that cancer is labeled as the 2nd leading cause of deaths in the United States.[2] Considering these statistical analysis, cancer carries the red flag and continues to be a crucial threat to public health. To combat against cancer, there is an utmost requirement to develop novel research methods to decrease the high mortality. Current combating approaches include prophylaxis, quick diagnosis, researching of new treatment strategies or the combinations of all.[3] The most deadly and prevalent form of malignant neoplasm is the carcinomas that is the major reason of mortality.[4] It is important to understand the initial events that are responsible for the migration and invasion of the carcinoma cell. In this particular review, we mainly focused on the advanced molecular research, to develop and design *de novo* treatment strategies for combating the different kinds of carcinoma, specially focused on Fascin and the expected outcome of the updated research in this regard.

Generally, most of the cancer patients die, not because of the tumor in the primary site, rather because of local invasion and/or distant metastasis.[5] Invasion, migration and metastasis of the cancer cells are the major causes of mortality. This is a complex multi-step process that causes alteration in the extra cellular matrix (ECM) and these changes lead in budding off the tumor cells from the primary site to distant organs.[6] 90% of the cancer patients die due to such metastasis.[7] Hence migration and invasion of the tumor cells are the key steps involved in the metastasis.[8] Local invasion (ability of the cancer cells to leave the primary tumor bed), Intravasation (entrance into blood vessels), Extravasation (escape from the circulation) and infiltration to distant tissues/ organs. Metastasis is highly coordinated process that depend on the changes in the cell to cell and cell to ECM and leads to dyna-mic changes in cell morphology.[9,10]

### Fascin:

Multicellular organisms have several classes of highly conserved actin-binding proteins that cross links various actin filaments to form tight bundles. These filaments, including stereocilia, microvilli, invadopodia and filopodia, and thus play a key role in different cytoskeletal processes. Fascin belongs to such class of actin-binding proteins and because of its actin filaments organizing ability, it was named fasciculus (Latin word) which means a small bundle.[11] Fascin is predominantly present in filopodia and is proved to have

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a crucial role in both normal and tumor cell migration. Fascin, a 55 kD globular protein, having four tandem fascin domains, each domain corresponds structurally to a β-trefoil fold.[12] Sea urchin coelomocytes was the first source from which Fascin was firstly obtained and later on in Drosophila as the singed gene product. [11,13] Both Drosophila echinoderms and melanogaster genomes encode a single form of fascin, while vertebrate genomes encodes 3 forms of Fascin i.e. fascin-1, widely expressed in nervous system and mesenchymal tissues; fascin-2, in retinal photoreceptor cells; and fascin-3 is testis specific.[14] Normally, Fascin is expressed in neuronal and mesenchymal cells and in epithelia its expression is low or absent.[15,14] For immunohistochemical analysis, tissue microarray technology was used on embryos, fetuses (4–22 weeks of gestation) and adult human specimens and the results showed that Fascin is expressed in the neural tube at 4 weeks of gestation. In the later developmental stages (8–12 weeks) and in adults, expression of homogenous gene was observed in the cells of the GIT and cerebellum. No expression was observed in Purkinje cells of the cerebellum and glandular epithelium of the GIT. Throughout in the development of lymphoid tissue (follicular dendritic cells), neurons, stratified squamous epithelia (basal layer cells), mesenchyme and vascular endothelial cells, Fascin is found to be expressed significantly. Negative Fascin expression has been found in simple columnar epithelia of the ovary, biliary duct, stomach, colon and pancreas. These data suggest that expression of Fascin is highly time and tissue specific.[16]

Figure 1 shows the x-ray crystal, biochemical and cryo-electron tomographic structure of Fascin describing that Fascin contains two major and important binding sites for actin. Conformational changes in the actin-binding sites, cryo-electron tomography-based reconstructions of the fascin/actin bundles and Fascin mutants x-ray crystal structure has provided the molecular basis for the Fascin mechanical properties and unique structure. All the four β-trefoil folds of fascin contributes to the two major Fascin actin-binding sites.[17]

Fascin forms filopodia (60 to 200 nm in diameter), which are straight and compact bundles formed by tight packing of 10 to 30 parallel actin filaments. Filopodia are finger like projections derived from plasma membrane and provides unique mechanical stiffness to the actin bundles.[18] Filopodial formation is high in the metastatic tumor cells and is directly proportional to the aggressiveness i.e. the more the numbers of filopodia, the more invasive and aggressive is the carcinoma.[19] Tan et al.[20] performed 26 immunohistochemical meta-analysis on cancer patients, the data showed that metastasis of lymph node, distant tissue metastasis, progression of the disease and increased risk of mortality is directly related to the high expression of fascin level. That is the reason that over expression of Fascin could be used as a new biomarker for early diagnosis and prognosis of metastatic and aggressive cancer. Recently, Increased level of Fascin expression has been reported in most of the transformed and metastatic tumors, including hormone receptor–negative breast carcinomas, squamous cell carcinomas of the esophageal, urothelial, gastric, ovarian, lung, skin and colonic carcinomas.[21-28]

**Advanced Strategies for the Inhibition of Fascin:**

Early diagnosis and development of new molecular and adjuvant therapeutics are the most important goal of today's research. As it is clear from the above discussion that expression of Fascin can be a novel biomarker for the aggressive metastatic tumors hence advanced research is needed to find innovative methods for its early detection and inhibition. For this purpose, advanced strategies used during recent researches and their possible outcomes are discussed hereunder:

**Interference or Silencing of RNA:**

Remarkable advancements have been made in the field of molecular biology, such as RNA interference (RNAi) or silencing of RNA. It is a specific post-transcriptional regulatory pathway, the outcome of which is to interfere/silent function of a specific gene. As a potential therapy, gene silencing has been widely used in clinical trials for a number of diseases.[29,30] High specificity, long lasting effect and efficient regulation of various isoforms of target genes, are its important advantages.[31] RNAi is a powerful tool to target gene of interest in cell culture and hence widely used in gene silencing experiment.[32] Small interfering RNA (siRNA) and short hairpin RNA, used as RNAi,[33,34] are discussed below in detail.

Chen et al.[35] introduced vector-based siRNA into OSCC cells for the down regulation of Fascin. Immunofluorescent microscopy indicated that the silencing/ interference of Fascin gene, results in decrease level of Fascin at protein level. It has a direct effect on cell surface protrusions causing the suppression of tumor cell invasion and migration. Zou et al.[36] found that RNAi effectively suppressed specific genes expression in early mouse embryonic cells as well as in undifferentiated embryonic stem (ES) cells. siRNA also known as short interfering RNA or silencing RNA is a double-stranded RNA composed of 20-25 base pair. Due to its complementary nucleotide sequences, interfere the expression of specific genes by degrading mRNA after transcription.

**Knockdown of Fascin through Short Hair-pin RNA (shRNA):**

A short hairpin RNA or small hairpin RNA (shRNA/ Hairpin Vector) is an artificial RNA molecule with a

tight hairpin turn that can be used to silence a target gene expression via RNAi.[37] Expression of shRNA in cells is typically established using transfection of plasmids or through viral or bacterial vectors. shRNA is an advantageous mediator of RNAi because it has a relatively low degradation rate, less turnover, achieving longer silencing and lower costs compared to synthetic siRNA.[38]

Hashimoto *at al.* induced shRNA for the silencing of Fascin in human colon carcinoma cells, which were obtained from primary aggressive tumor.[39] Knockdown of Fascin changes cells protrusion morphology and hence resulted in decrease filopodia formation, adhesion and Rac-dependent migration. In vivo studies showed decreased xenograft tumor development and metastasis. Through shRNA using wild-type green fluorescent protein, fascin and mutated forms of fascin at the protein kinase C (PKC) phosphorylation site were knockdown. Results indicated that both actin-bundling and active PKC-binding activities of fascin are crucial for filopodial protrusions, tumor metastasis and Rac-dependent migration. In another study Yang et al.[40] designed 4 different shRNA vectors for the knockdown of Fascin. After successful transfection of the shRNA, the vector with best interference effect on Fascin was chosen and used in trans-well migration and scratch assays. The outcome of the results showed that the migration capacity of bone marrow mesenchymal stromal cells (BM-MSCs) in vitro is due to Fascin. Chen et al.[41] found significant differences in Fascin expression after and before the knockdown of Fascin through shRNA.

From these studies, it is concluded that Fascin is the active contributor for the invasion and migration of different tumor cells in vitro and in vivo tumor development and metastasis. Briefly, the outcomes of these research suggested that, 1) Fascin has specific contribution in migration of different carcinomas by affecting the assembly of filopodia, related protrusions and the disassembly of focal adhesions, 2) Rac is involved for the upregulation of Fascin activity and 3) both the actin-bundling and PKC-binding activities are needed to promote tumor metastasis for fascin. Most importantly, the xenograft tumor experiments also showed that Fascin is the active contributor in primary tumors development and in their metastatic spread. These results give a foundation for considering Fascin as a carcinoma biomarker and potential therapeutic target as well. In future knockdown of Fascin through shRNA can be important to stop the phosphorylation as well as the aggressiveness of the metastatic tumors and to develop therapeutic knockdown mechanisms in various human carcinomas.

**Ectopic Expression of MicroRNAs (miR-NAs):**

MicroRNAs (miRNAs) consisting of 21-23 nucleotides, is a class of endogenous non-coding RNAs that is conserved evolutionarily. It primarily binds to the 3'-untranslated region (UTR) of the target mRNAs complementary sequence region and thus inhibit translation or causes destabilization of specific mRNAs.[42] miRNA dysfunction is involved in many biological processes such as cancer development and progression. FSCN1 miRNAs is regulated in many carcinomas and dysregulation of these miRNAs in different tumorigenesis act as oncogenes or tumor suppressors.[43,44]

A co-relation between the expression level and functions of miR-539 in hepatocellular carcinoma (HCC) was determined through multiple molecular analyses. The results indicated that miR-539 is significantly down-regulated in HCC cells and tissue samples. miR-539 ectopic expression inhibited cell viability, proliferation, invasion and migration in vitro and also suppresses tumor xenograft growth in vivo. FSCN1 over expression promotes HCC cell invasion and migration, which is confirmed as a direct target of miR-539. Thus in the development of HCC and its progression, miR-539 acts as a novel tumor suppressor that target FSCN1. These results provided new insight suggesting that miR-539 may be a therapeutic target.[6] miR-539 has few verified targets that can inhibit invasion and migration in various types of carcinomas, including thyroid cancer, prostate cancer and osteosarcoma.[45,46,5]

The conclusion is that miRNAs are essential hallmarks for different carcinomas and their dysregulation has been identified as a key for monitoring, early diagnosis, prognosis and treatment of the deadly cancer. Target Scan, microRNA.org and RNA hybrid databases can be used to screen and choose specific miRNAs that could inhibit Fascin in different carcinomas. There are various evidences that indicates that Fascin will be a direct downstream target for various miRNAs, because, 1) Fascin at the 3'-UTR contains highly conserved nucleotide sequences that will be complementary to the miRNA seed sequence, 2) From the study of different carcinomas, it has been clear that levels of miRNAs are substantially reduced compared to the normal adjacent tissues. So this review highlights a new correlation between miRNA and Fascin in various carcinomas.

**Blockage of Tumor Cell Migration and Metastasis by using Fascin Inhibitors:**

Yang et al.[17] studied systematic mutagenesis of 100 fascin mutants and identified at least two major actin binding sites on fascin molecule, as shown in Fig 1.Each of these actin binding sites is very important for filopodial protrusions in tumor cells. Impairment of any one of these individual actin-binding sites demolishes fascin actin-bundling activity. In tumor metastasis, one of the important steps is the migration of tumor cell from its primary site, which is not possible without actin cytoskeletal reorganization. Filopodia is an antenna like

protrusive structures, which are formed due to actin binding and bundling activities and Fascin has a key role in this activity. Han et al. idealized and developed small fascin specific inhibitory molecules that are specific to inhibit Fascin and actin filament interaction. These inhibitors cause the inhibition of Fascin binding and bundling activities in vitro, and cancer cell migration and metastasis in vivo. These inhibitors get attach to one of the two Fascin specific actin-binding sites and thus cause the blockage of the other actin-binding site as well. Thus reduces the overall binding activity of actin filaments, results in the inhibition of Fascin bundling activity and impairments of actin cytoskeletal reorganization at the cellular level. Chen et al.[48] used migrastatin analogues as a potent inhibitors for cancer cells invasion, migration and metastasis. Migrastatin is a natural product which are synthesized and secreted by *Microbe Streptomyces*.[1,2] From the crystalline X-ray structure it is revealed that these migrastatin analogues specifically bind to one of the Fascin binding sites and inhibit its activity.

More advance research is needed to formulate more improved fascin inhibitors that would inhibit both Fascin actin-binding and bundling activities with the main aim to impair actin cytoskeletal reorganization for the formation of lamellipodial, filopodial, and stress fiber formation. These will ultimately stop cancer cell invasion, migration and metastasis to decrease the aggressiveness of various carcinomas. So improved fascin inhibitors could be of great clinical importance and a new strategy for the treatment of various metastatic tumor to decrease its aggressiveness.

**Monoubiquitination:**

Ubiquitination is the addition of ubiquitin molecule to a protein substrate, whose main function is to impair protein synthesis process in many ways. Numerous biological processes are control through Ubiquitination by regulation of protein interaction, degradation and localization etc.[49] and has been reported in DNA repair regulation, virus budding, endocytosis and nuclear export.[50] Lin et al.[51] found that Fascin dysregulation has been involved in cancer cell metastasis. They idntified that monoubiquitination is a new mechanism for the regulation of Fascin binding and bundling acti-vity. K247 and K250 were identified to be the sites for monoubiquitination and at the positive charge patch of the actin binding site 2 (ABS2), two Fascin residues were identified. Through chemical ubiquitination method, monoubiquitinated Fascin (mUbfascin) is syn-thesized and its effect on both Fascin actin bundling and dynamics activity was determined. Monoubiquiti-nation decreased Fascin bundling, increased the time of bundle assembly initiation and accelerate the existing bundles disassembly. Lin et al.[52] explored the underline molecular mechanism that is involved in the regulation of Fascin bundling activity through monoubiquitination. To find out the post-translational modification of Fascin, immunoprecipitation (IP) and LC-MS/MS was used while for the synthesis of monoubiquitination a novel chemical monoubiquitination method was used. The monoubiquitinated Fascin was then purified and the effect on fascin bundling activity was determined using low speed sedimentation assay, transmission electron microscopy and fluorescence microscopy. Using chemically synthesized monoubiquitinated Fascin (mUb-fascin), the effects of monoubiquitination on fascin bundling activity and dynamics were investigate. Monoubiquitination introduces steric hindrance to interfere the interaction between the positively charged patch at ABS2 and actin filaments. Mutation of the monoubiquitination sites (K247 and K 250) to arginine inhibited fascin monoubiquitination and enhanced pro-migration ability of fascin, supported the notion that monoubiquitination inhibit fascin activity.

**Antimicrobial Peptides (AMPs):**

Antimicrobial peptides (AMPs) are small (< 10 kDa) amphipathic and cationic peptides of variable lengths, structure and are essential part of the most living organism against invading immunogen. From the last couple of decades, AMPs are isolated from different sources like neutrophils, macrophages, haemocytes, epithelial cells, etc. of animals (vertebrates and invertebrates), plants, fungi and bacteria.[53] AMPs are primitive immune defense mechanism, found in a wide range of eukaryotic organisms ranging from humans to plants to insects,[54] exhibiting varying degree of antimicrobial properties against virus, Gram-positive and negative bacteria, parasites and fungi.[55] That's why AMPs are being considered as a potential candidate for novel therapeutic purposes.

Natural killer (NK)-lysin is a member of the saposin-like protein family, which is a cationic (9-kD) protein isolated originally from porcine intestinal tissue.[56] It is an effect or peptide of cytotoxic T lymphocytes and NK cells, found to kill a variety of tumor cells and have no effect on erythrocytes. [57,58] Our lab has successfully expressed and purified recombinant porcine natural killer lysin (rpNK-lysin) in *Pichia pastor is* system and tested its *in vitro* anticancer activity. The results indicate that rpNK-lysin has a potent antitumor activity against SMMC-7721 cell line with negligible hemolytic activity against human erythrocytes. Compared with the control group, rpNK-lysin treated cells showed marked morphological alterations with decrease filopodia numbers and growth.[59] Moreover, recently our lab has explored that hepatocellular carcinoma cells (SMMC-7721, 97-H and HepG2) proliferation, adhesion, invasion and metastatic properties are suppersed by rpNK-lysin by inhibiting Fascin1 expression.[60,61] Thus, the recombinant porcine NK-lysin could

be developed as a potentially therapeutic agent for tumor growth inhibition.

## CONCLUSIONS AND FUTURE PERSPECTIVES

Marvelous work has been done in the recent years to understand, prevent and manage the deadly malignant diseases but still cancer remains a leading risk for worldwide mortality and morbidity. Current molecular approaches against fighting this disease comprises of preventive tools, early diagnosis and numerous therapeutic strategies. Nevertheless, the role of Fascin protein in the metastatic tumor is very crucial; still it needs some more advancement and its applicableness to the human field to combat the deadly metastatic tumors. The conclusion of this thorough review and the findings raise a number of important and fruitful suggestions about Fascin pathophysiology and its implementation for routine clinical uses and treatment in patients that has been diagnosed for major carcinomas. Although the prognostic markers are very much important and needed but the utmost importance is to combat the deadly cancer today and in the long future through early diagnosis. Early diagnosis is the hidden key for the effective treatment of cancer patients to cure and give life to many patients with cancer and also necessary to control the aggressiveness of the metastatic tumors. As Fascin is not a membrane-bound or secreted protein, it is likely to be served as a serological protein biomarker for many carcinomas examined.

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## AUTHOR'S CONTRIBUTION

**AK:** Conception of work and design.
**KF:** Acquisition of data and substantial contribution and design.
**HLH:** Drafting article and receiving critically.

## CONFLICT OF INTEREST

None to declare.

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