

Potential of metastin and metastin receptor as biomarkers for urological cancers

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Abstract

AIM: To investigate the current state of the research of metastin and metastin receptor in the urological cancer field.

METHODS: For analyzing the value of metastin and metastin receptor as molecular biomarkers for the patients with urological cancer, MEDLINE database searches were performed using these terms: metastin, KISS1, kisspeptin, renal (cell) carcinoma (RCC), kidney cancer or urothelial cancer or bladder cancer or prostate cancer or testicular cancer (tumor). Since the articles were evaluated by the validity of the articles

based on plausibility, credibility, and evidence levels, the articles were graded according to their level of evidence, using the grading system defined by the Oxford Centre for Evidence-based Medicine.

RESULTS: A total of six clinical studies published by individual institutions between 2003 and 2013 were included in this review. The article numbers for each of the evidence levels 2a and 2b were three (50%) and three (50%), respectively. Immunohistochemistry and reverse transcriptase-polymerase chain reaction using tumor tissues were performed to analyze in five articles (83%) and in one article (17%). The value of metastin and/or metastin receptor as molecular biomarkers in clear cell RCC, upper tract urothelial carcinoma, and bladder cancer was evaluated by multivariate analysis. Low expression of metastin receptor in clear cell RCC and low expression of metastin in upper tract urothelial carcinoma were significant risk factors for metastasis, and low metastin expression was an independent prognostic factor in bladder cancer.

CONCLUSION: Metastin and metastin receptor have potential as suitable molecular biomarkers for urological cancers. However, future studies of metastin and metastin receptor should undergo external validation to ensure consistency across different patient series, since individual institutional studies lack generalization.

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Key words: KISS-1; Metastin; Metastin receptor; Metastasis; Renal cell carcinoma; Upper tract urothelial carcinoma; Bladder cancer

Core tip: Metastin and metastin receptor have attracted interest in the field of cancer because of their novelty and potential to inhibit cancer metastasis. Furthermore, they have potential as suitable molecular biomarkers for urological cancers. However, the results of all of

the studies analyzed in this review were retrospectively obtained. Therefore, future studies of metastin and metastin receptor should undergo external validation to ensure consistency across different patient series, since individual institutional studies lack generalization.

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INTRODUCTION

Cancer metastasis is a leading cause of death in cancer patients. Metastasis is a complex, multistage process in which malignant tumor cells spread from the primary tumor to secondary organs. Tumor cells acquire an invasive phenotype to invade the stromal tissue and disrupt the vascular endothelium. Once in the blood, the disseminated tumor cells (DTCs) must survive in the circulating environment and escape physical damage and attack by the immune system. After the tumor cells arrest or adhere to vessel walls, they invade through the capillary wall (extravasation)^[1,2]. Finally, DTCs must adapt to the new microenvironment of the secondary site and start to form micrometastasis or reprogram to a quiescent state, which can last for years^[1,2]. The metastasis suppressor genes are defined by their ability to prevent the development of metastasis by inducing apoptosis or dormancy once the cells have lodged at the secondary site. The proteins encoded by these genes participate in a diverse range of signaling pathways, and in some cases they inhibit not just one but multiple steps in the metastatic cascade^[2]. The *KISS-1* cancer metastasis suppressor gene is located on human chromosome 1q32^[3], and encodes a carboxy-terminal amidated peptide with 54 amino acid residues called metastin (kisspeptin), which was identified as the ligand of a G-protein-coupled receptor, the metastin receptor^[4]. Metastin and metastin receptor inhibit tumor invasion or migration through focal adhesion kinase, paxillin, MAP kinase or RhoA, and have been implicated in melanoma, thyroid cancer, esophageal squamous cell carcinoma, hepatocellular carcinoma, pancreatic carcinoma, breast cancer, ovarian cancer, renal (cell) carcinoma (RCC), upper tract urothelial carcinoma, bladder cancer, and prostate cancer^[5-7]. Furthermore, metastin and metastin receptor were shown to be expressed in the hypothalamus, brain stem, spinal cord, pituitary, ovary, prostate and placenta in normal human tissue, and they play a pivotal role in the control of the hypothalamic pituitary-gonadal axis *via* regulation of gonadotropin-releasing hormone secretion^[8].

The objective of this review article was to investigate the value of metastin and metastin receptor in urological cancers.

MATERIALS AND METHODS

To analyze the clinical study of metastin and/or metastin receptor, MEDLINE database searches were performed using the following terms: metastin, *KISS-1*, kisspeptin, renal (cell) carcinoma or kidney cancer, urothelial carcinoma or bladder cancer or prostate cancer or testicular cancer. Since the articles were evaluated by the validity of the articles based on plausibility, credibility, and evidence levels^[9], the articles were graded according to their level of evidence using the grading system defined by the Oxford Centre for Evidence-based Medicine^[10].

RESULTS

Current state of the literature for metastin and metastin receptor in urological cancer

A total of six articles published between 2003 and 2013 were included in this review. The majority of the data were predominantly obtained *via* nonrandomized, retrospective, but often controlled studies. Immunohistochemistry (IHC) and reverse transcriptase-polymerase chain reaction analyses of tumor tissues were performed in five articles (83%)^[6,11-14] and in one article (17%)^[15]. Thus, the article numbers for each of the evidence levels^[10] 2a and 2b were three (50%) and three (50%), respectively (Table 1).

Summary of the clinical studies of metastin and metastin receptor in urological cancer

Renal cell carcinoma: Metastin receptor was reported as being more highly expressed in RCCs compared to normal tissue^[11,16]. Chen *et al*^[11] reported that lack of metastin receptor was correlated with rapid progression of clear cell RCC. In an IHC study of 131 patients with clear cell RCC, the absence of metastin receptor was significantly associated with a poor overall or tumor-specific survival in Kaplan-Meier survival analysis ($P < 0.0001$)^[11]. Furthermore, 121 patients with clear cell RCC exhibited positive staining of low to high metastin receptor expression, and the remaining 10 patients with negative immunostaining died because of tumor progression. However, the expression of metastin had no correlation with tumor-specific survival ($P = 0.778$)^[11]. Shoji *et al*^[12] reported that lack of metastin receptor is a predictor of metastasis after radical nephrectomy for pT1 clear cell RCC. IHC analysis of samples from 54 patients with clear cell RCC revealed that the sensitivity, specificity, positive predictive value, and negative predictive value with negative immunostaining of metastin receptor were 85.7%, 97.6%, 46.2%, and 97.6%, respectively^[12]. Metastasis-free survival rates were significantly higher in patients with positive staining (97.6%) than in patients with negative staining (53.8%) ($P < 0.001$)^[12]. In univariate analysis for metastasis-free survival, negative immunostaining of metastin receptor was a significant risk factor for metastasis ($P = 0.001$)^[12]. Furthermore, negative immunostaining of metastin receptor was an independent predictor for metastasis in multivariate analysis ($P = 0.002$)^[12].

Table 1 Summary of metastin and/or metastin receptor literature in the urological cancer field

	Author (yr)	Patients (n)	The methods of the analyses (sample type)	Evidence level ^[14]
Renal cell carcinoma	Chen <i>et al</i> ^[11]	131	IHC (tumor tissue)	2a
	Shoji <i>et al</i> ^[12]	54	IHC (tumor tissue)	2a
Urothelial carcinoma	Takeda <i>et al</i> ^[6]	151	IHC (tumor tissue)	2b
Bladder cancer	Sanchez-Carbayo <i>et al</i> ^[13]	69	IHC (tumor tissue)	2a
	Nicolle <i>et al</i> ^[14]	64	IHC (tumor tissue)	2b
	Cebrian <i>et al</i> ^[15]	205	RT-PCR (tumor tissue)	2b

Level 2a: Systematic review (with homogeneity) of either retrospective cohort studies or untreated control groups in randomized controlled trials; Level 2b: Retrospective cohort study or follow-up of untreated control patients in a randomized controlled trial; or derivation of a clinical decision rule or validated on split-sample only. RT-PCR: Reverse transcriptase-polymerase chain reaction; IHC: Immunohistochemical analysis.

Upper urinary tract urothelial carcinoma: Takeda *et al*^[6] reported that 44 patients (29.1%) had distant metastasis during follow-up; 39.1% (27 of 69 patients) with low metastin expression compared with 20.7% (17 of 82 patients) with high metastin expression in an IHC study of 151 patients with upper urinary urothelial carcinoma. Univariate analysis revealed that low metastin expression, pT2 or greater, and positive lymphovascular invasion were significant risk factors for metastasis^[6]. In multivariate analysis, low metastin expression ($P = 0.028$), pT2 or greater ($P = 0.013$), and positive lymphovascular invasion ($P < 0.001$) were independent predictors for metastasis^[6]. The 5-year metastasis-free survival rates were 60.9% for the patients with low expression of metastin and 81.0% for patients with high expression of metastin ($P = 0.012$)^[6].

Bladder cancer: Metastin was reported to be highly expressed in bladder cancers^[13,14]. Sanchez-Carbayo *et al*^[13] reported that metastin expression was significantly associated with stage ($P = 0.031$) and not with tumor grade. In an IHC study of 69 patients with bladder cancer, mean survival time and median survival time of patients with expression lower than 20% was 14.7 and 9.0 mo, respectively^[13]. Furthermore, mean survival time and median survival time of patients with expression higher than 20% was 47.3 and 37.0 mo, respectively^[13]. Nicolle *et al*^[14] reported that metastin receptor was expressed at high levels in bladder cancers compared with normal bladder tissue, and the difference between low- and high-grade groups was significant ($P = 0.03$). However, there was no association between the expression of metastin and grade^[14]. The researchers suggested that the expression of metastin receptor is highly deregulated in invasive and high-grade tumors more often than in superficial and low-grade tumors^[14]. Cebrian *et al*^[15] reported that upregulated metastin expression was found in low-grade and early lesions, compared with high-grade ($P = 0.010$) and invasive bladder tumors ($P = 0.001$). They reported that tumors with metastin methylation had lower transcript expression than unmethylated tumors ($P = 0.037$), and low transcript levels of metastin were significantly associated with increasing stage ($P < 0.0005$) and grade ($P = 0.024$)^[15]. In a series of 205 patients with bladder cancer, high expression of metastin indicated favorable outcomes. Furthermore, multivariate analysis indicated that metastin

expression and tumor stage were independent prognostic factors ($P = 0.001$), with hazard ratios of death of 2.62 (95%CI: 1.49-4.58; $P = 0.001$) and 0.42 (95%CI: 0.20-0.85; $P = 0.017$), respectively^[15].

DISCUSSION

In the current review, the value of metastin and/or metastin receptor as molecular biomarkers in clear cell RCC^[12], upper tract urothelial carcinoma^[6], and bladder cancer^[13] were evaluated in a multivariate analysis of clinical studies. Low expression of metastin receptor in clear cell RCC and low expression of metastin in upper tract urothelial carcinoma were found to be significant risk factors for metastasis^[6,12], and low expression of metastin was an independent prognostic factor in bladder cancer^[15]. However, the results showed that the data in all of the studies were obtained retrospectively. An increasing number of molecular biomarkers have been investigated by numerous teams, with inherent differences regarding population size, demographics, techniques used, and interpretation of results. However, many negative findings have not been published as a result of a lack of enthusiasm by researchers to declare negative findings and because of the reluctance of scientific journals to publish negative reports, thus lending bias to the overall field. Therefore, all studies of molecular biomarker including metastin and metastin receptor should undergo external validation to ensure consistency across different patient series, since individual institutional studies lack generalization^[17].

Metastin receptor was found by Ohtaki *et al*^[4] as a rat orphan receptor (rOT7T175) that was nearly identical to GPR54 during a search for novel G-protein-coupled receptors using a degenerate polymerase chain reaction strategy. To identify the endogenous ligand of a human orphan receptor, these authors established a stable CHO cell line expressing the human counterpart metastin receptor (CHO/h175). Although hOT7T175 has 39.2% amino-acid identity to human galanin receptor GALR2, the cells did not show any response to a panel of known peptides, including galanin and galanin-like peptides. However, human placental extract induced a robust increase in the intracellular calcium ion concentration ($[Ca^{2+}]_i$) in CHO/h175 cells. The amino-terminal sequence obtained for the isolated peptide was identical to

the partial amino-acid sequence (Gly 68 to Ala 88) of the *KISS-1* gene product. This sequence was isolated from human placenta as the endogenous ligand of an orphan G-protein-coupled receptor and was termed “metastin”^[4]. For the function of metastin as a metastasis suppressor protein, excessive formation of focal adhesion and stress fibers by phosphorylation of focal adhesion kinase and paxillin in cells expressing metastin receptor might be one of the mechanisms through which tumor metastasis is inhibited by metastin^[4,18-20]. Takino *et al.*^[21] reported that metastin forms a complex with pro-matrix metalloprotease (MMP) and active MMP-2, while MMP-9, matrix type (MT) 1-MMP, MT3-MMP and MT5-MMP cleave the Gly118-Leu119 peptide bond of both full-length metastin and metastin decapeptide. MMP plays an important role in development and morphogenesis by participating in extracellular matrix re-modeling^[22]. Cancer cells also use MMP for invasion and metastasis. Invading cells are forced to proliferate within an embedded dense three-dimensional matrix composed largely of type I collagen or cross-linked fibrin^[23-26]. Moreover, digestion of the metastin decapeptide by MMP abolished its ligand activity. Takino *et al.*^[21] proposed that: (1) metastin is used as an antimetastatic agent in combination with MMP inhibitor; or (2) MMP-resistant forms of metastin are developed that may also be efficacious.

The mechanisms of metastin and metastin receptor as metastasis suppressor proteins in urological cancers were hypothesized^[6,13,16]. In RCC, metastin induced excessive formation of focal adhesions in RCC cell lines, which are located at the ends of stress fibers in RCC cell lines^[16]. The results of these experiences suggested that metastin regulates focal adhesion and metastasis through the Rho-GTPase route by activating one or several of its members^[16]. In urothelial carcinoma, metastin significantly reduced the invasiveness of a bladder cancer cell line and inhibited the DNA-binding activity of nuclear factor kappa B by blocking its nuclear translocation, leading to a reduction in the expression and activity of MMP-9^[6]. In another study, metastin function was considered as an upstream regulator of E-cadherin^[13]. Furthermore, metastin was aberrantly silenced by CpG island hypermethylation in bladder cancer cell lines^[15]. In bladder cancer, the increased methylation rate together with the loss of transcript expression of *KISS-1* was also found to be associated with increasing tumor stage and poor clinical outcome^[15].

Wang *et al.*^[7] reported the potential of metastin as a molecular biomarker for predicting prognosis in patients with prostate cancer. *In vitro*, metastin inhibited the invasion of a prostate cancer cell line, PC-3M. Furthermore, IHC staining showed weak or lack of metastin expression in prostate cancer tissue^[7]. Although no significant difference in metastin expression was observed between primary and metastatic tissues ($P = 0.3$), loss of metastin expression was positively correlated with clinical stages II / III, IV and metastatic tumors ($P < 0.01$), and metastin expression was significantly lower in metastatic tissues

than in earlier stage primary prostate cancer ($P < 0.01$), indicating that loss of metastin expression correlated with prostate cancer progression^[7]. Moreover, the expression of metastin receptor was weak and only mildly positive in normal prostate tissues, and decreased expression was observed in primary and metastatic tissues^[7].

In conclusion, metastin and metastin receptor have attracted interest in the field of cancer because of their novelty and potential to inhibit cancer metastasis. Furthermore, they have potential as suitable molecular biomarkers for urological cancers. However, the results of all of the studies analyzed in this review were retrospectively obtained. Therefore, future studies of metastin and metastin receptor should undergo external validation to ensure consistency across different patient series, since individual institutional studies lack generalization.

COMMENTS

Background

Cancer metastasis is a leading cause of death in cancer patients. Metastasis is a complex, multistage process in which malignant tumor cells spread from the primary tumor to secondary organs. The *KISS-1* cancer metastasis suppressor gene is located on human chromosome 1q32, and encodes a carboxy-terminal amidated peptide with 54 amino acid residues called metastin (kisspeptin), which was identified as the ligand of a G-protein-coupled receptor, the metastin receptor. Metastin and metastin receptor inhibit tumor invasion or migration through focal adhesion kinase, paxillin, mitogen-activated protein kinase or RhoA, and have been implicated in melanoma, thyroid cancer, esophageal squamous cell carcinoma, hepatocellular carcinoma, pancreatic carcinoma, breast cancer, ovarian cancer, renal (cell) carcinoma (RCC), upper tract urothelial carcinoma, bladder cancer, and prostate cancer.

Research frontiers

In the current review, the value of metastin and/or metastin receptor as molecular biomarkers in clear cell RCC, upper tract urothelial carcinoma, and bladder cancer were evaluated in a multivariate analysis of clinical studies.

Innovations and breakthroughs

Low expression of metastin receptor in clear cell RCC and low expression of metastin in upper tract urothelial carcinoma were found to be significant risk factors for metastasis, and low expression of metastin was an independent prognostic factor in bladder cancer.

Applications

Metastin and metastin receptor have attracted interest in the field of cancer because of their novelty and potential to inhibit cancer metastasis. Furthermore, they have potential as suitable molecular biomarkers for urological cancers.

Peer review

This paper is meaningful because a description of metastin and metastin receptor was clarified as suitable molecular biomarkers for urological cancers.

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