Review paper

The aberrant overexpression of vimentin is linked to a more aggressive status in tumours of the gastrointestinal tract

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Abstract

Vimentin is an intermediate filament protein normally expressed in cells of mesenchymal origin, e.g. myofibroblasts, chondrocytes, macrophages, and endothelial cells. The expression of vimentin, which has been thought of as the main mesenchymal marker, is also detected in tumour tissue. In tumours of the gastrointestinal tract vimentin expression is usually correlated with advanced stage of tumour, lymph node metastasis, and patient survival.

This article is dedicated to Professor Ewa Siekierska.

Introduction

Vimentin is a 57 kDa intermediate filament protein normally expressed in cells of mesenchymal origin, e.g. myofibroblasts, chondrocytes, macrophages, and endothelial cells [1]. Under physiological conditions, vimentin plays a significant role in cell adhesion, probably by regulating the structure of $\alpha 6\beta 4$ integrin. In the case of exogenous laminin absence, integrin $\alpha 6\beta 4$ is assembled to basal fibrillar structures by an intercellular mechanism involving the $\beta 4$ cytoplasmic tail and plectin. Upon binding to laminin, integrin $\alpha 6\beta 4$ undergoes redistribution to hemidesmosome-like structures, which show associations with vimentin filaments. This mechanism may play pivotal role in maintaining endothelial barrier integrity [2]. It should also be noted that vimentin is the major class of intermediate filaments detected in leukocytes, playing an essential role both in the attachment of lymphocytes to the vascular endothelium and in the transcellular migration of lymphocytes through endothelial cells [3]. In recent years, a great number of studies have linked intermediate filaments to signalling pathways. They can act as signalling platforms and scaffolds for different types of signalling molecules [4]. This is exemplified by the ability of vimentin to regulate Erk signalling. Some studies have demonstrated that the cleavage fragments of de novo synthesised vimentin have the ability to interact with phosphorylated Erk1 and Erk2 MAP kinases (pErkr) in injured sciatic nerves [5]. The pull-down and ELISA experiments showed robust calcium-dependent binding of pErk to the second coiled-coil domain of vimentin. It should be also noted that binding competition experiments with pErk peptides confirmed a solution in which vimentin covers the phosphorylation lip in pErk interacting with residues above and below the lip. The same peptides inhibited pErk binding to the dynein complex in sciatic nerve axoplasms and interfered with protection from phosphatases by vimentin. Thus, a soluble intermediate filament fragment may interact with signalling kinases [6].

The expression of vimentin, which has been thought of as the main mesenchymal marker, is also detected in tumour tissue [4]. The loss of E-cadherin and the gain of mesenchymal markers such as vimentin and fibronectin are demonstrated as hallmarks of epithelial-mesenchymal transition (EMT), which is a critical process in met-

astatic cascade. This is because during EMT, cells detach from the main tumour mass enter the blood stream and invade the surrounding tissue [7–9]. The increased expression of vimentin has been reported in various tumour cell lines and tissues including prostate cancer, breast cancer, and endometrial cancer. Expression of this protein has also been documented in tumours of the central nervous system, malignant melanoma, and tumours of the gastrointestinal tract and is usually correlated with advanced stage of disease and poor clinical outcome [4].

In this paper we will review the role of vimentin, expression of which contributes to the aggressive phenotype and poor prognosis in gastrointestinal cancers.

Vimentin expression in tumours of the gastrointestinal tract

As mentioned above, vimentin is overexpressed in various tumours, including tumours of the gastrointestinal tract. One of the most significant global health problems, despite a decline in incidence and mortality, is still gastric cancer. About 95% of gastric cancers are caused by adenocarcinoma originating from the glandular cells of the stomach lining. There are two main types of gastric adenocarcinoma: intestinal and diffuse. The intestinal type of gastric cancer is characterised by cohesive neoplastic cells which form gland-like tubular structures. In contrast, the histology of diffuse gastric cancer is characterised by poorly differentiated cells and no glandular structures [10]. In the case of gastric cancer, vimentin expression was observed in patients with advanced stage of cancer, especially in the group with macroscopically scirrhous-type of gastric carcinoma. In other cases, expression of this protein was closely related to the diffuse type of disease, lymphatic invasion, and lymph node metastasis [11]. Very similar results have been obtained by Ryu *et al.*, who have also reported that expression of vimentin in gastric cancer tissues is correlated with lymph node metastasis, vascular and neural invasion, and advanced stage of tumour. Moreover, the results of Kaplan-Meier univariate analysis demonstrated that vimentin expression status predicted disease-free survival (DFS) but did not affect overall survival (OS) [12]. It is also worth mentioned that in gastric cancer vimentin expression is closely correlated with the expression of Smad interacting protein 1 (SIP1). Results of real-time reverse transcription (PCR) analysis demonstrated that the patients showing high levels of vimentin mRNA and vimentin protein had a tendency toward poorer prognoses than those characterised by a low level of vimentin expression. It was especially observed in the group of intestinal type of gastric cancer patients with the high level of SIP1 expression [13]. This thesis is supported by a study with the use of MKN7 intestinal type of gastric cancer cell line. Knockdown of SIP1 in those cells impaired cellular proliferation, migration, and invasion [13]. Iwatsuki *et al.* revealed that circulating vimentin positive cells can survive in peripheral circulation, then undergo EMT in the bone marrow and implant at metastatic sites. It is not surprising then, that in gastric cancer patients, cells with expression of vimentin are also present in the bone marrow. Moreover, the level of vimentin mRNA expression in the bone marrow increased concordantly with the clinical staging and was clearly correlated with tumour invasion and lymph node metastasis [14]. Otsuki *et al.*, examining samples of gastric cancer, reported that strong immunoreactivity of vimentin was detected in the stromal cells of diffuse-type gastric cancer. This high expression of vimentin was closely related to decreased expression of E-cadherin [9, 15]. Which factors are responsible for decreased levels of E-cadherin expression during tumour progression? It is probably caused by factors including SIP1, slug, and twist, which bind to E-boxes within the CDH1 (E-cadherin) promoter [15]. Therefore, tumour samples with high expression of these proteins show lower expression of E-cadherin.

Vimentin expression was associated with significantly higher incidence of lymph-node metastasis also in the case of oesophageal squamous cell carcinoma (ESCC). This type of cancer is the eighth most common lethal malignancy in the world, despite advances of surgical techniques and incorporation of new therapeutic approaches. The 5-year survival rate is about 40% [16–18]. The ESCC patients characterised by high expression of vimentin had significantly worse prognosis, which is connected with advanced tumour status and lymphatic invasion [19].

As it has been widely reported that the third most commonly diagnosed cancer in the world is colorectal cancer. Ten-twenty percent of patients with stage II colorectal cancer, and about 40% with stage III, are thought to develop recurrence of disease [8, 20]. Vimentin expression in colon cancer samples is a useful tool in identifying patients with poor prognosis, but it should also be noted that in colon cancer tissue expression of vimentin was detected mainly in stromal cells and in lymphocytes within both normal colonic crypts [21]. Overall survival in the group characterised by high vimentin, expression in tumour stroma was about 71%, in comparison to 90% in the group with low expression. In this context, it is worth noting that increased stromal vimentin expression indicated dynamic changes in the tumour stroma during tumour progression, e.g. fibroblastic changes, appearance of new vessels, and appearance of lymphocytes – tumour-infiltrating lymphocytes (TIL).

All of these components are characterised by vimentin expression. In the case of colon cancer, the prognostic power of vimentin expression (risk ratio = 3.5) was better than that of lymph node metastasis (risk ratio = 2.2) [22]. Vimentin immunostaining is also observed in rhabdoid colorectal tumour (RCT), which is a rare, highly aggressive neoplasm recurrent in elderly patients, commonly at the caecum. In patients with RCT, vimentin may be of clinical value to make a differential diagnosis to predict poor outcome or to choose the best-fit therapy [23].

Expression of vimentin is also detected in hepatocellular cancer (HCC), which is the primary tumour of the liver, developing in the setting of cirrhosis or precirrhotic chronic liver injury [24]. The long-term prognosis for patients undergoing curative hepatic resection is poor with 5-year survival rate from 20% to 53%. Tumour grade, microvascular invasion, and the presence of microsatellite lesions have been thought to predict HCC patient survival [25, 26]. Moreover, in these patients the level of E-cadherin and vimentin expression also predicted survival. In this case, vimentin expression level was clearly correlated with shorter disease-free survival (DFS) and overall survival (OS). Moreover, statistical analyses demonstrated also the correlations between vimentin expression and poor tumour differentiation, vascular invasion, or extrahepatic recurrence following curative surgery. During development of HCC metastasis a pivotal role may be played by zinc-finger-enhancer binding protein 1 (ZEB1). In MHCC-97HshZEB1 cells (after ZEB1 silencing) increased level of epithelial E-cadherin and decreased level of vimentin have been observed [27]. The migration assay showed that after 24-hour incubation, the number of migrated MHCC-97H-shZEB1 cells was significantly less than that of migrated MHCC-97Hcontrol. Downregulation of ZEB1 gene may suppress the motility of metastatic HCC cells and the same the expression of vimentin [28]. Very similar results have been obtained in a study with the use of colon cancer cells. In this case, ZEB1 downregulation was responsible for reduction in vimentin activity. At the same time, upregulation of E-cadherin expression and secretion of laminin-5 to the extracellular matrix was detected. As a consequence, decreased motilities of cells have been reported [29]. The role of vimentin during HCC progression was also studied by the use of highly invasive Sk-Hep-1 cells. The migratory abilities of those cells were inhibited by Vim-silencing. After this silencing, downregulation of MMP-9 activities were shown [30]. It is worth mentioning that MMP-9 is an enzyme of the Metzian family, which takes part in pro-oncogenic mechanisms such as neoangiogenesis, tumour cell proliferation, and metastasis. MMP-9 degrades type IV collagen and cleaves pro-cytokines, chemokines, and growth factors, which are very important during tumour progression.

In pancreatic ductal adenocarcinoma (PDAC) vimentin expression is correlated with poor histological differentiation. This cancer, despite an extensive clinical and scientific effort, is fourth on the list of cancer-related causes of death. At the time of diagnosis, most patients are at advanced stage of disease, and only about 20% of them qualify for surgical resection. After this the 5-year survival rate is about 20–30% and vimentin expression in neoplastic cells is an indicator of shorter survival [31, 32]. Hong *et al.* revealed that pancreatic cancer tissue had a threefold higher level of vimentin expression than colon, lung, and ovarian tumours. Interestingly, a more specific antigenic form of vimentin (MW 53.3 kDapI 5.1) has been expressed at a 5–10 higher level in pancreatic tumours in comparison to the tumours of lung or colon. Furthermore, this expression was approximately 50% higher than that observed in pancreas without any pathological changes [33].

As demonstrated above, vimentin plays a crucial role in the development and progression of gastrointestinal cancers but also serves as a potential diagnostic marker to predict patient survival. Expression of vimentin is mainly characterised during the EMT process, and it seems that some events in the metastatic process such as cell migration and invasion are consequences of vimentin overexpression in tumour cells. However, it should be noted that in some cancers, e.g. colon cancer, vimentin expression is detected in stromal cells. Nevertheless, also in this case the presence of vimentin predicted survival of patients and was connected with worse prognosis. A number of studies have addressed the functions of intracellular vimentin, while the role of extracellular vimentin still remains unclear. Understanding the mechanisms that are connected with the functioning of this class of vimentin, especially with vimentin gene regulation, may contribute to better understanding of the invasiveness of cancer cells. In recent years, vimentin protein has been considered as a promising candidate as a target for cancer therapy. The use of vimentin-specific chemical inhibitors, antibodies, aptamers, or siRNA, in combination with other agents, may be very important from a clinical point of view and should be encouraged. In particular, identification of vimentin-specific aptamers would be of great value, because aptamers exhibit high binding specificity and can be modified. In comparison to antibodies, these molecules are relatively small (10–20 kDa), and interestingly they show reduced immunogenicity. Therefore, it is very important to check the vimentin expression profile in different types of gastrointestinal cancers with special emphasis on its isoform and localisation.

After this, it will be possibly to develop novel cancer treatment options.

Vimentin methylation as a common alteration in gastrointestinal cancer development

Increased DNA methylation is an epigenetic alteration that is common in cancer tissue. Aberrantly methylated DNA has been reported as a potential tumour marker [4]. The study by Chen *et al.* defined a DNA sequence within vimentin exon 1, which is generally targeted for aberrant DNA methylation by human colon cancers, especially those that arise in the proximal colon. This aberrantly methylated exon has been reported in faecal DNA as a marker detecting the presence of neoplastic cells in nearly half of colon cancer patients. Moreover, testing for vimentin gene methylation in faecal DNA showed high levels of specificity – approximately 90%. It is also worth noting that detection of vimentin exon 1 methylation in faecal DNA with primer set 29 has the same sensitivity in the detection of colon cancers, which arise proximal to the splenic flexure (46% sensitivity) and those arising distal to the splenic flexure (45% sensitivity) [34]. With use of quantitative methylation-specific PCR (qMSP) Shirahata *et al.* reported that vimentin methylation is characteristic for advanced colorectal cancers with liver metastasis and peritoneal dissemination. No significant correlations were detected between the presence of aberrant methylation and patient gender, maximal tumour size, extent of tumour, or lymph-node metastasis [35]. Vimentin methylation also occurs frequently in the case of gastric adenocarcinoma [4]. The highly differentiated adenocarcinomas were significantly methylated in comparison to poorly differentiated ones. However, no correlations were found between the presence of aberrant methylation in gastric carcinoma samples and TNM stage (the extent of tumour, spread to the lymph nodes, and distal metastasis). Thus, the aberrant methylation can be potentially used for the detection and monitoring of gastric carcinoma in clinical samples such as serum, because, firstly, the method of qMSP has a high level of sensitivity. Secondly, as it has been reported in the study by Kitamura *et al.* that vimentin gene methylation occurs frequently in gastric carcinoma tissues [36].

In summary, vimentin expression is common in tumours of the gastrointestinal tract and is usually related to poor clinical outcome. In some cases it may be of clinical value to choose the best-fit biological therapy. Also, vimentin methylation might be a promising marker for detection of tumour DNA in the serum of gastrointestinal patients.

Conflict of interest

The authors declare no conflict of interest.

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