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REVIEW

Measuring midkine: the utility of midkine as a biomarker in cancer and other diseases

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Midkine (MK) is a pleiotropic growth factor prominently expressed during embryogenesis but down-regulated to neglible levels in healthy adults. Many published studies have demonstrated striking MK overexpression compared with healthy controls in various pathologies, including ischaemia, inflammation, autoimmunity and, most notably, in many cancers. MK expression is detectable in biopsies of diseased, but not healthy, tissues. Significantly, because it is a soluble cytokine, elevated MK is readily apparent in the blood and other body fluids such as urine and CSF, making MK a relatively convenient, accessible, non-invasive and inexpensive biomarker for population screening and early disease detection. The first diagnostic tests that quantify MK are just now receiving regulatory clearance and entering the clinic. This review examines the current state of knowledge pertaining to MK as a biomarker and highlights promising indications and clinical settings where measuring MK could make a difference to patient treatment. I also raise outstanding questions about reported variants of MK as well as MK's bio-distribution *in vivo*. Answering these questions in future studies will enhance our understanding of the significance of measured MK levels in both patients and healthy subjects, and may reveal further opportunities for measuring MK to diagnose disease. MK has already proven to be a biomarker that can significantly improve detection, management and treatment of cancer, and there is significant promise for developing further MK-based diagnostics in the future.

LINKED ARTICLE
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Abbreviations
AFP, α-fetoprotein; AKI, acute kidney injury; CEA, carcinoembryonic antigen; FDA, US Food and Drug Administration; HCC, hepatocellular carcinoma; HSPGs, heparan sulphate proteoglycans; IHC, immunohistochemistry; *Mdk*, midkine gene; MK, midkine protein; MM, malignant mesothelioma; NAG, *N*-acetyl-D-glucosaminidase; N-GAL, neutrophil gelatinase-associated lipocalin; RA, rheumatoid arthritis

Introduction

Midkine (MK) is a heparin-binding growth factor first discovered as a highly expressed gene (*Mdk*) during mouse embryogenesis (Kadomatsu *et al.*, 1988). Early studies of MK focused on its role in embryonic development (Kadomatsu *et al.*, 1990; Nakamoto *et al.*, 1992; Uehara *et al.*, 1992; Muramatsu *et al.*, 1993; Satoh *et al.*, 1993), but subsequently MK expression was observed in adult organisms as well (Muramatsu, 1993). Overexpression of the MK protein has now been widely observed in association with human diseases, particularly cancer. MK's biological functions and roles in disease have been extensively reviewed recently elsewhere (Muramatsu, 2011; Weckbach *et al.*, 2011; Sakamoto and Kadomatsu, 2012; Kadomatsu *et al.*, 2013). Furthermore, because MK is a soluble, secreted cytokine, it can be readily quantitated in blood samples, thereby making it a minimally invasive biomarker for detecting, monitoring and managing illness. This paper reviews the current knowledge of MK expression in healthy and diseased patients, evaluates MK's usefulness as a biomarker, and highlights the diagnostic tests that are reaching the clinic. Promising specific disease indications where measuring MK has strongest potential to assist in managing disease are also suggested. Finally, this review also raises some key remaining questions about MK variants and bio-distribution. Addressing these questions is likely to generate further opportunities for using MK as an effective biomarker to manage disease.

Gene and tissue expression of MK in healthy subjects

In healthy adults, *MDK* gene expression has been detected at a variety of sites, including the gastrointestinal tract, the kidney, the spleen, the lungs and the thyroid, with Northern blotting indicating strongest relative *MDK* expression to be in the mucosa of the small intestine (Tsutsui *et al.*, 1993). Two sites strikingly lacking any *MDK* expression are the healthy liver (Tsutsui *et al.*, 1993; Aridome *et al.*, 1995; Koide *et al.*, 1999), and healthy lung tissue (Garver *et al.*, 1993). In the instances where *MDK* may be detectable in some healthy tissues, quantitative PCR shows that this expression is typically weak, and many fold below that of malignant tissue (Aridome *et al.*, 1995; O'Brien *et al.*, 1996; Ye *et al.*, 1999; Moon *et al.*, 2003; Huang *et al.*, 2007; Jia *et al.*, 2007). Interestingly, although *MDK* gene expression may be evident in some healthy tissues, there is a distinct absence of any detectable corresponding MK protein expression (Miyashiro *et al.*, 1997; Huang *et al.*, 2007). In studies on healthy tissue that investigated both *MDK* gene expression and MK protein expression, employing anti-MK antibodies in immunohistochemistry (IHC) from the same healthy tissue samples has reported a clear absence of MK in, for instance, the oral cavity (Ruan *et al.*, 2007), salivary glands (Ota *et al.*, 2010), stomach (Huang *et al.*, 2007; Zhao *et al.*, 2012), colon (Ye *et al.*, 1999) and pancreas (Maeda *et al.*, 2007).

In contrast, the one site where MK protein expression consistently occurs in healthy adults, at least in mice, is the kidney (Muramatsu, 1993), where MK is evident in proximal and distal tubular epithelial cells (Sato *et al.*, 2001) and endothelial cells (Kato *et al.*, 2011). No comprehensive corresponding IHC studies have been published examining healthy human tissue, but some support of this hypothesis comes from faint MK staining seen in biopsies of non-cancerous kidney, although it must be noted that these samples did also contain minor glomerular abnormalities (Kosugi *et al.*, 2007). In summary, although *MDK* gene expression has been detected at a number of tissue sites in healthy subjects, most of the circulating MK protein is apparently synthesized by the kidneys (Sakamoto and Kadomatsu, 2012).

Circulating MK in healthy subjects

MK is a soluble growth factor, lacking any sort of membrane-spanning domain. As such, it is secreted by the cells that produce it, and this MK reaches the circulation. Because there is some MK production ongoing even in healthy subjects, this results in a healthy 'background' level of peripheral blood MK. As a first step to understanding the utility of measuring MK as a disease biomarker, it is essential that healthy normal MK ranges are established.

As yet, no large-scale population studies have been conducted to comprehensively establish normal and reference MK ranges. However, a number of published studies have quantified the circulating MK concentration in small cohorts of apparently healthy adults, mostly for use as control groups in cancer studies. Table 1 summarizes the healthy control MK levels and ranges from a survey of the MK literature undertaken for this review. Fifteen publications were deemed eligible for inclusion, based on their reporting of MK serum or plasma data from healthy control samples. Studies reporting data for only very low numbers of healthy controls ($n < 10$) or studies where descriptive statistics were not sufficiently reported were excluded. Validation data from a commercially available MK ELISA kit for 99 healthy subjects (Figure 1A) have also been included in the analysis (Cellmid, 2013) because of the public availability of normal distribution data for this product (http://midkine.cellmid.com.au/kit-documents). The development and performance of this ELISA test has been described in part recently elsewhere (Sommerschuh *et al.*, 2012).

Taken together, the 15 studies in Table 1 (totalling 1512 healthy subjects) show a clear uniformity of blood MK levels and ranges. This is striking, given the wide variety of subjects studied (ethnicity, age), the sample matrices tested (serum vs. plasma), the differences in the reagents used (polyclonal vs. monoclonal antibodies, variety of sources of MK for standard curve generation), and the various tests employed ('home brew' vs. commercial ELISA kits). This uniformity suggests MK has significant 'robustness' as a circulating biomarker, and that the MK levels measured are not prone to large fluctuations in value due to the vagaries of different testing methods. The distribution of healthy MK values falls predominantly between 0 and 625 $pg \cdot mL^{-1}$, with zero MK being uncommon and approximately 95% of values below 625 $pg \cdot mL^{-1}$ (Tables 1 and 2). Occasional individuals record MK levels exceeding 1000 $pg \cdot mL^{-1}$. One plausible explanation for the rare cases where MK exceeds 1000 $pg \cdot mL^{-1}$ is that these subjects are in fact asymptomatic individuals with an as yet undetected underlying disease, particularly cancer or an inflammatory or autoimmune disorder. The so-called healthy control samples in all of the studies in Table 1 rely on anonymous blood donations from volunteers. Generally, such blood donors are pronounced as 'healthy' on the basis of a relatively superficial medical assessment (often a brief questionnaire alone). In such circumstances, a donor with unaddressed disease might easily pass for inclusion in a healthy control cohort. However, none of the studies in Table 1 report any follow-up of the 'healthy' controls with elevated MK levels, so one can only speculate on the reasons behind their increased values.

Table 2 shows a crude meta-analysis of the 15 studies included in Table 1, giving a putative indication of where the anticipated normal population mean, median and frequency distribution might lie. A schematic representation of this data is shown in Figure 1B. There is a striking similarity between the anticipated normal population frequency deduced from Tables 1 and 2 and the actual distribution reported for analytical validation of the commercially available MK test (Figure 1A). As such, Figure 1A,B both give a reasonable estimate for what the true healthy MK range and distribution is likely to be for the population at large.

Summary of this section

Normal circulating MK concentrations have been reported in a number of different studies using a variety of reagents,

Table 1

Studies reporting MK serum or plasma concentrations for healthy human subjects

Study	Subjects *n (F:M)* Age range (years) Nationality	Serum or plasma	Mean/median MK conc (pg·mL^{-1}) (±SD) (25–75%)[a]	Highest MK value measured[a] (pg·mL^{-1})	95% cut-off[b] (pg·mL^{-1})	Mean ± 2 SDs (pg·mL^{-1})
Ikematsu *et al.*, 2000; Shimada *et al.*, 2003[c]	Healthy adults 135 (94:41) 21–75 Japanese	Serum	Mean: 154 (±76) Median: nr (nr)	490*	306	306
Soulie *et al.*, 2004	Healthy adults 30 (21:9) 20–59 French	Plasma	Mean: 10 Median: 15 (nr)	100	nd	nd
Obata *et al.*, 2005	Healthy adults 275 (133:142) Mean age: 54 Japanese	Serum	Mean: 190 (±146) Median: 170 (81–273)	nr	nd	482
Salama *et al.*, 2005	Healthy elderly adults 32 (24:8) 61–84 Japanese	Serum	Mean: nr Median: 500 (385–520)	580	nd	nd
Jia *et al.*, 2007	Healthy adults 26 (2:24) 18–65 American	Serum	Mean: nr Median: 12 (4–42)	110*	nd	nd
Krzystek-Korpacka *et al.*, 2007	Healthy adults 42 (10:32) 25–56 Polish	Serum	Mean: nr Median: 130 (nr)	1000*	563	nd
Ota *et al.*, 2008	Healthy adults 134 (61:73) 20–92 Japanese	Serum	Mean: 491 (±98) Median: nr (nr)	780*	<600	687
Ibusuki *et al.*, 2009	Healthy adults 104 (48:56) nr Japanese	Plasma	Mean: 489 Median: 489 (411–542)	1068	<751	nd
Kemik *et al.*, 2010	Healthy adults 38 (16:22) 37–71 Turkish	Serum	Mean: 360 (±100) Median: nr (nr)	nr	nd	560
Krzystek-Korpacka *et al.*, 2010	Healthy adults 108 (64:44) 21–66 Polish	Serum	Mean: nr Median: 93 (nr)	395*	350*	nd
Rice *et al.*, 2010	Healthy adults 61 (61:0) 32–69 Australian	Plasma	Mean: nr Median: 383 (~220–800)*	~8000*	1095*	nd
Rawnaq *et al.*, 2011	Healthy adults 148 (50:98) 27–70 German	Serum	Mean: 128 (±115) Median: 99 (33–198)	nr	nd	358
Zhu *et al.*, 2013	Healthy adults 210 nr Chinese	Serum	Mean: nr Median: 195 (nr)	1860*	840*	nd
Krzystek-Korpacka *et al.*, 2013	Healthy adults 70 (25:45) Polish	Serum	Mean: 245 Median: nr (nr)	1050*	680*	nd
Cellmid, 2013	Healthy adults 99 (49:50) 42–62 German	Serum	Mean: 209 (±108) Median: 191 (132–254)	648	427	425

[a]In instances where an exact value has not been supplied by the authors, but the individual data point is plotted on a graph, values have been estimated by reading off graphs. Estimates obtained in this manner are indicated by *. [b]Value below which 95% of individuals tested fall. [c]Both studies report the same healthy control group. nd, not determinable; nr, not reported.

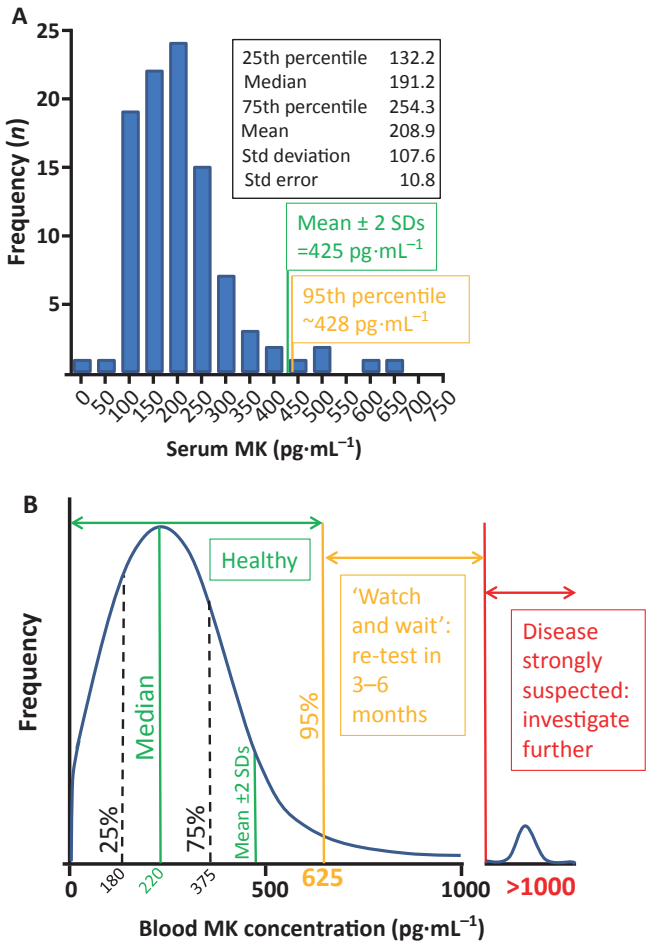

Figure 1

Frequency distribution of MK concentration in the blood of normal healthy subjects. (A) Actual serum data from 99 healthy blood donors tested during the analytical validation of a commercially available MK ELISA (Cellmid, 2013). These validation data produce a frequency distribution with very similar statistical parameters to those anticipated from the meta-analysis of the published MK cancer studies in Tables 1 and 2. (B) Schematic illustration of the probable normal population frequency distribution for blood MK. The anticipated median, 25th and 75th percentiles are taken from Table 2. Approximately 95% of individuals would be expected to record blood MK below 650 pg·mL^{-1}, and mean ± 2 SDs would occur around 470 pg·mL^{-1}. In a population screening test where only MK was measured, subjects with blood MK under 650 pg·mL^{-1} would be considered 'healthy'. Blood MK >1000 pg·mL^{-1} would be considered suspicious, with more specific or definitive (e.g. imaging) testing recommended as immediate follow-up. Subjects with MK levels between 650 and 1000 pg·mL^{-1} would be considered for active surveillance, with re-testing in 3–6 months to determine if MK levels were increasing.

samples and tests. Despite the diversity of approaches, the studies show broad agreement as to a normal MK range, with approximately 95% of individuals having blood MK below ~625 pg·mL^{-1}. These collated data establish a reasonable, anticipated, healthy normal range for researchers to use in future studies of normal versus disease levels of MK.

MK overexpression in disease

By far the most extensively studied disease for overexpression of MK is cancer, and MK's immediate utility as a biomarker lies predominantly in oncology. However, it is also clear that MK can be elevated in inflammatory, autoimmune and ischaemic conditions as well.

Oncology

MK overexpression has been reported for at least 20 different cancer types (Table 3) for malignancies in all major organs and tissue types, ranging from the most common cancers to some of the rarest. As such, MK can truly be considered a 'pan-cancer' biomarker. Overexpression of both the *MDK* gene and the MK protein within the tumour is a typical feature of cancer. Furthermore, where MK overexpression is a feature of a particular tumour type, this manifests as an elevated circulating MK concentration. As Table 3 shows, in every instance of tissue MK overexpression in which that blood MK has been investigated, circulating MK is also elevated. There are no instances in which a tumour type with elevated tissue MK did not also show increased circulating MK. Therefore, it seems that circulating MK measurement is likely to be an accurate 'proxy' of tumour MK expression in any given tumour type, making biopsy unnecessary in MK evaluation. This is an advantage for any biomarker as, compared with biopsy, sampling blood is minimally invasive, convenient, relatively cheap and can be performed frequently if required.

A recent systematic review (Krzystek-Korpacka and Matusiewicz, 2012) evaluated the published studies on circulating MK in cancer including those in Table 3. Krzystek-Korpacka *et al*. found four important features common to the cancer studies of circulating MK:

- MK levels were significantly elevated in cancer compared with healthy normal samples.
- MK levels were significantly elevated in cancer when compared with non-malignant diseases of the same tissues.
- MK levels generally increased with increasing severity of cancer.
- Where tumours were surgically resected, circulating MK levels usually decreased immediately afterwards, and would often increase again if the cancer recurred.

Many tumour types that show elevated MK already have established biomarkers that are routinely tested during diagnosis, treatment and monitoring. Often, these biomarkers have been in use for decades and they have become the default clinical markers by virtue of familiarity, even when they are widely known to be inadequate. For example, the primary blood biomarker used to monitor recurrence of colorectal cancer (CRC) is carcinoembryonic antigen (CEA). In use for over 40 years, CEA is the marker recommended by the American Society of Clinical Oncologists for monitoring metastatic CRC recurrence after treatment, yet CEA elevation only occurs for 55–75% of patients with recurrent metastatic CRC at most (Sorbye *et al*., 2012; Su *et al*., 2012). In

Table 2

Meta-analysis of the healthy control studies reported in Table 1

MK concentration (pg·mL^{-1}) *(Number of studies)*[a]				
Mean of the means	**Mean of the medians**	**Mean of the 25–75% ranges**	**Mean of the 95% cut-off**	**Mean of the mean ± 2 SDs**
253 *(9)*	208 *(11)*	181–376 *(7)*	623 *(9)*	470 *(6)*

[a]Data taken only from studies where the variables have been reported.

Table 3

Midkine gene and protein overexpression in various cancers in tissue, blood and urine

	Midkine overexpression relative to healthy tissue						
	Tissue		Blood		Urine		
Cancer type	***Mdk* gene**	**MK protein**	***Mdk* gene**	**MK protein**	***Mdk* gene**	**MK protein**	**References**
Breast	✓	✓	✓	✓		✓	Miyashiro *et al.*, 1997; Ikematsu *et al.*, 2000; 2003; Ibusuki *et al.*, 2009
Ovarian	✓			✓			Nakanishi *et al.*, 1997; Rice *et al.*, 2010
Uterine/cervical	✓	✓		✓			Moon *et al.*, 2003
Prostate	✓						Konishi *et al.*, 1999
Lung	✓	✓		✓			Ikematsu *et al.*, 2000
Neuroblastoma		✓		✓			Nakagawara *et al.*, 1995; Fiegel *et al.*, 2008; Ikematsu *et al.*, 2008; Lucas *et al.*, 2009
Glioblastoma	✓	✓					Mishima *et al.*, 1997; Stylianou *et al.*, 2009
Meninginoma	✓	✓					Tong *et al.*, 2007
Neurofibromatosis type 1	✓	✓		✓			Mashour *et al.*, 1999; 2004
Gastric	✓	✓		✓		✓	Rha *et al.*, 1997; Ikematsu *et al.*, 2003; Obata *et al.*, 2005; Zhao *et al.*, 2012
GI stromal		✓		✓			Kaifi *et al.*, 2007; Rawnaq *et al.*, 2011
Bladder	✓	✓		✓	✓	✓	O'Brien *et al.*, 1996; Ikematsu *et al.*, 2003; Fiala *et al.*, 2006; Konety, 2006; O'Sullivan *et al.*, 2012
Colorectal	✓	✓		✓		✓	Ikematsu *et al.*, 2003
Duodenal	✓	✓		✓			Ikematsu *et al.*, 2000
Oral squamous cell	✓	✓		✓			Jham *et al.*, 2011
Oesophageal squamous cell	✓	✓		✓			Ikematsu *et al.*, 2000; 2003; Shimada *et al.*, 2003
Hepatocellular	✓	✓		✓		✓	Ikematsu *et al.*, 2000; 2003; Jia *et al.*, 2007
Bile duct	✓	✓		✓		✓	Ikematsu *et al.*, 2000; 2003
Pancreatic	✓	✓		✓		✓	Ikematsu *et al.*, 2000; 2003; Maeda *et al.*, 2007; Ohhashi *et al.*, 2009
Renal						✓	Ikematsu *et al.*, 2003
Thyroid	✓	✓		✓		✓	Ikematsu *et al.*, 2000; 2003
Osteosarcoma	✓	✓					Maehara *et al.*, 2007; Sueyoshi *et al.*, 2011
Chronic lymphocytic leukaemia				✓			Cohen *et al.*, 2011

Blank field denotes not yet determined. GI, gastrointestinal.

comparison, MK was elevated in all cases of metastatic CRC, and MK strongly outperformed CEA at detecting CRC at all disease stages in a comparative study of MK and CEA (Krzystek-Korpacka *et al.*, 2013). Other tumour types where MK has outperformed the currently used blood biomarker test include the hepatocellular carcinoma (HCC) marker, α-fetoprotein (AFP) (Jia *et al.*, 2007; Zhu *et al.*, 2013), and the oesophageal squamous cell carcinoma markers CEA and cytokeratin 19 fragment (CYFRA21-1) (Shimada *et al.*, 2003).

However, despite the apparent superiority of MK over these established circulating biomarkers, it is extremely difficult to displace these markers in standard clinical practice. The reasons for this are discussed in the section 'Utility of MK as a biomarker'.

Ischaemic disease

One of MK's most significant biological functions in adults is to preserve tissue viability under hypoxic stress, including that induced during ischaemia (Muramatsu, 2011). MK expression is initiated in occluded tissues during ischaemic events in the heart (Horiba *et al.*, 2006), brain (Yoshida *et al.*, 1995; Mochizuki *et al.*, 1998), kidney (Sato *et al.*, 2001) and limbs (Horiba *et al.*, 2000). Presumably, endogenous expression of MK occurs to protect tissue as administration of MK reduces apoptosis and limits tissue death in the infarct area (Fukui *et al.*, 2008; Ishikawa *et al.*, 2009; Ishiguro *et al.*, 2011). MK also conveys longer term benefits in tissue recovery through angiogenesis (Fukui *et al.*, 2008; Takenaka *et al.*, 2009; Weckbach *et al.*, 2012). As with tumour-derived MK, the MK expressed during ischaemic events reaches the circulation and is manifested as increased blood MK concentration (Kitahara *et al.*, 2010). In a prospective study of consecutive hospital admissions for treatment of heart failure, serum MK levels at admission were higher in patients with heart failure than in controls and MK levels independently predicted adverse clinical outcomes including death (Kitahara *et al.*, 2010). As such, measuring serum MK may be of value as a prognostic or predictive biomarker in cardiac ischaemia, and maybe also in other ischaemic indications such as stroke and limb thrombosis.

Kidney injury

It is clear that increased renal expression of MK is strongly associated with, and a key initiator of, kidney injury and damage due to a variety of causes (Sato *et al.*, 2001; Kawai *et al.*, 2004; Kosugi *et al.*, 2007; Kato *et al.*, 2011; Kosugi and Sato, 2012; Salaru *et al.*, 2013). This gives a strong *prima facie* case for investigating utility of MK as a biomarker in kidney injury and disease. However, this review only uncovered one study that evaluated (urinary) MK levels in renal disease (Hayashi *et al.*, 2009). In this study, MK strongly outperformed three other urinary biomarkers (*N*-acetyl-D-glucosaminidase, NAG; neutrophil gelatinase-associated lipocalin, N-GAL; and IL-18) in diagnosing established acute kidney injury (AKI). MK was also elevated earlier than NAG, N-GAL and IL-18 during abdominal aortic aneurysm surgery, suggesting MK has further utility as an early marker of the induction of kidney injury.

Autoimmune disease

In animal models, MK expression plays a significant role in inflammatory and autoimmune diseases, including in disease models of rheumatoid arthritis (RA) (Takada *et al.*, 1997; Maruyama *et al.*, 2004) and multiple sclerosis (MS) (Liu *et al.*, 1998; Wang *et al.*, 2008; Sonobe *et al.*, 2012). Limited human data show that MK is elevated in the synovial fluid and sera of RA patients. There are no published studies of MK expression in MS patients. Further basic studies investigating MK elevation in human subjects are required to determine whether MK offers utility as a biomarker in RA and MS.

One study has shown that serum MK has potential utility as a biomarker of Crohn's disease, particularly (Krzystek-Korpacka *et al.*, 2010).

Other diseases

MK elevation has been investigated in limited studies for a range of other diseases. Circulating MK was overexpressed in sepsis and septic shock patients, but MK levels did not differentiate between survivors and non-survivors (Krzystek-Korpacka *et al.*, 2011). Circulating MK levels were also significantly higher in Alzheimer's disease patients compared with healthy controls (Salama *et al.*, 2005).

Utility of MK as a biomarker

The discovery and application of over- (or under-) expressed genes and proteins for detecting or diagnosing disease ('biomarkers') has long been a focus of medical science. The first cancer biomarker, free Ig light chain found in the urine of multiple myeloma patients ('Bence Jones protein'), was reported more than 150 years ago (Jones, 1848). Subsequently, many hundreds of potential biomarkers have been published and reported, and particularly since the early 2000s with the advent of the human genome sequence and the growth and development of high-throughput systems for scanning whole genomes and proteomes, biomarker discovery has burgeoned. However, the vast majority of these biomarkers remain clinically unused, or at best highly experimental, in medicine. The reason for this is that any new biomarker or diagnostic test must meet two fundamental criteria to be clinically useful:

1 *Its measurement must allow clinicians to make a different (better) clinical decision to the one they would otherwise have taken.*
2 *The different clinical decision taken should result in a meaningful clinical outcome for the patient* (e.g. longer overall survival, longer progression-free survival or reduced treatment side effects).

There are three barriers that make approval of novel biomarkers to this standard difficult. Firstly, sufficient demonstration of points 1 and 2 typically requires prospective clinical studies that measure ultimate patient outcomes (such

as survival), and not merely retrospective studies on stored patient samples to demonstrate improved sensitivity and/or specificity compared with current tests. This means a properly conducted biomarker study is akin to a therapeutic clinical trial in length, but probably with greater patient numbers. An alternative approach is to conduct blinded 'prospective–retrospective' clinical studies on previously collected patient samples. However, such studies require that appropriate material has been archived properly and is available; this is rare. Secondly, even in cases where a biomarker demonstrates utility in a prospective-style study, clinician professional bodies are typically very conservative in their evaluation of novel biomarkers. Before recommending a change to clinical practice, they demand extremely high standards of evidence, often from very large patient numbers in multiple studies. Finally, the requirements by regulators such as the US Food and Drug Administration (FDA) and the European Medicines Agency are inconsistent and often unclear, further complicating the path of a new biomarker to becoming a product on the healthcare market. Together, these factors conspire to make successful development and approval of new biomarker tests relatively rare, especially for a single biomarker measured in isolation. These difficulties are well described in an excellent recent review (Hayes *et al.*, 2013), and they apply to MK just as much as to other experimental biomarkers.

A further challenge to the use of MK as a diagnostic biomarker in isolation is its generality, as the elevation of blood MK is not specific to a particular disease. Even within oncology, elevated MK alone cannot differentiate between tumour types. One strategy that increases the likelihood of clinical success for a non-specific biomarker such as MK is to measure it in conjunction with other biomarkers (either known or novel). Measuring combinations of biomarkers simultaneously ('multiplexing') in a single clinical sample has become technically and commercially feasible thanks to the many new platform technologies now available (Ling *et al.*, 2007). The results for each biomarker included in a multiplex test are typically processed using mathematical algorithms (e.g. see (Mamtani *et al.*, 2006; Centola *et al.*, 2013) to produce a single diagnostic readout. This approach to diagnostic development has gained acceptance from the US FDA (Boja *et al.*, 2011), and a number of multiplex tests are now on the market. MK is included in three multiplex cancer diagnostic tests that are currently seeking regulatory approval and entering the US market.

Bladder cancer recurrence: urine test

Pacific Edge Biotechnology (Dunedin, New Zealand) gained FDA registration and entered the US market in 2013 with its CX bladder test for detecting the recurrence of bladder cancer. Bladder cancer is one of the most commonly recurring cancers after treatment, and the current standard of care requires frequent disease surveillance via flexible tube cystoscopy, coupled with urinary cytology. Cystoscopy incurs significant false negatives (Konety, 2006), and is invasive, painful, intimate and costly (Abogunrin *et al.*, 2011). Cytology has high specificity but low sensitivity, so is not useful as a diagnostic test in its own right (Abogunrin *et al.*, 2011). MK was initially identified as a promising protein biomarker in serum and urine for detecting bladder cancer (Fiala *et al.*, 2006). The CX bladder test simultaneously measures urinary mRNA expression levels for four biomarkers including MK, and is the only non-invasive test that can match the accuracy of cytology (O'Sullivan *et al.*, 2012).

Early lung cancer detection: blood test

Celera (Alameda, CA, USA) has developed a six-biomarker blood test, including MK, for the detection of early stage lung cancer in at-risk subpopulations (Birse *et al.*, 2011). More than 500 proteins were assessed initially by Celera to select the final six-biomarker panel. A recent large-scale prospective screening trial in the United States demonstrated that thoracic CT imaging reduced lung cancer mortality by 20.3% over 5 years among high-risk individuals (smokers and ex-smokers) when compared with chest X-ray surveillance (Aberle *et al.*, 2011). However, any lesions identified by CT scan still require confirmatory biopsy, which incurs significant cost and morbidity. Further, approximately 25% of lesions biopsied were found to be non-cancerous. Celera's assay could replace biopsy with a less invasive, less debilitating, safer and less costly alternative to clarify the diagnosis of patients with suspicious pulmonary nodules. Celera has successfully adapted and validated this assay from ELISA format to the high-throughput multiplex Luminex bead platform (Tomic *et al.*, 2012).

Early stage malignant mesothelioma (MM) diagnostic: blood test

SomaLogic (Boulder, CO, USA) had developed and validated a panel of 13 blood-based protein biomarkers, including MK, that can detect MM at early stages of disease (Ostroff *et al.*, 2012). Over 1000 proteins were assessed during the assay development. Because MM is a relatively rare but deadly disease, even in high-risk groups (asbestos-exposed workers), there is a strong need of a highly specific test for risk surveillance and early detection that can be given frequently and is cost effective. Currently, many subjects exposed to asbestos are not screened for MM at all and those who are screened undergo expensive CT or X-ray imaging, with associated issues of radiation exposure. The SomaLogic blood test could replace imaging and be used to increase screening coverage of the at-risk population.

The successful development of these multiplexed diagnostic tests provides a template for how MK might be incorporated in further multiplex tests for managing other diseases. Strong opportunities include cancer, as currently many cancers are diagnosed and monitored by the measurement of a single protein biomarker alone, for example, prostate cancer antigen (PSA) for prostate cancer, CEA for CRC and AFP for HCC. In such cases, multiplexing the currently used biomarker with MK (and perhaps further biomarkers as well) offers an obvious avenue to improving test performance.

HCC

Whole-genome microarray screening of over 200 patients revealed *Mdk* to be one of the top five overexpressed genes in HCC, with serum MK protein being similarly overexpressed (Jia *et al.*, 2007). This provides an obvious opportunity to multiplex MK with the other most notably overexpressed candidates, either as a protein or as a gene test. Furthermore, a recent study of over 900 HCC patients demonstrated that MK alone greatly outperformed the current serum biomarker AFP in detecting early HCC (Zhu *et al.*, 2013). MK alone also has clear value for HCC disease monitoring and prognosis in already diagnosed patients (Hung *et al.*, 2011). In a head-to-head comparison with the currently used biomarker AFP, serum MK levels were significantly elevated even in instances of HCC where AFP levels were normal (Jia *et al.*, 2007).

CRC

In direct comparative testing of circulating MK with CEA testing for detecting CRC, MK was apparently superior to CEA in sensitivity (Krzystek-Korpacka *et al.*, 2013). Both MK and CEA are pan-cancer antigens; therefore, neither is suitable for definitively diagnosing CRC at initial patient presentation. However, MK is potentially a better blood marker for monitoring treatment and recurrence than CEA, and MK would also add value to multi-marker biomarker panels (Krzystek-Korpacka *et al.*, 2013).

Prostate cancer

MK is frequently overexpressed in cancerous, but not healthy, prostate tissue (Konishi *et al.*, 1999). MK overexpression may also indicate the more clinically serious androgen-independent form of the disease (Nordin *et al.*, 2013). Further studies are needed to confirm that prostate tissue overexpression translates into concomitant increases in circulating (and urine) MK concentrations. If so, one attractive opportunity for measuring MK in prostate cancer may be to use pair testing of blood MK with PSA. With billions of tests performed over the past 20 years, the PSA blood test is probably the most widely employed cancer screening and diagnostic test to consist of a single biomarker used on asymptomatic subjects. However, recent meta-analyses of the clinical benefits from PSA testing have found marginal or no benefit (Loeb *et al.*, 2011; Scheerer *et al.*, 2012; Ilic *et al.*, 2013; Lee *et al.*, 2013), and professional bodies in many countries now recommend against the use of PSA for routine screening (Heidenreich *et al.*, 2011; Lin *et al.*, 2011; Moyer, 2012). Of particular concern is evidence that PSA testing has led to over-treatment (Lee *et al.*, 2013), whereby patients with benign cancer have had biopsies and surgical resections – interventions with frequent significant and permanent harms (Ilic *et al.*, 2013). One suggested way to improve utility of PSA is to test other cancer biomarkers alongside PSA (Heidenreich *et al.*, 2013). Measuring MK in conjunction with PSA might achieve this; for example, perhaps only patients with both high PSA and MK velocities would be considered for further immediate intervention, whereas patients with high PSA values alone would be put on active surveillance.

AKI

AKI is a significant morbidity in a number of diverse clinical settings, including multi-organ trauma, surgery, imaging and chemotherapy. Convenient, rapid measures to detect AKI early in its onset would be of great clinical advantage in such conditions, because interventions could then be taken to protect the kidneys. Biomarkers to effectively manage AKI remain elusive, firstly due to the heterogeneous causes of the condition, and secondly because highly time-sensitive biomarkers are needed (Devarajan, 2011). The traditionally used blood (creatinine, blood urea nitrogen) and urinary markers (protein casts and creatinine) of kidney function lack this time sensitivity (Han *et al.*, 2008). Multi-marker panels are now being tested for early and rapid AKI diagnosis, with some success (Luo *et al.*, 2013), but the use of MK either as an adjunct test or as a part of the panel offers opportunities to further improve test performance (Hayashi *et al.*, 2009).

Cardiac ischaemia

Traditionally, heart failure has been risk stratified by assessing the cause of failure (e.g. coronary artery atherosclerosis), pathophysiological characteristics (e.g. systolic heart failure), and the acuity and severity of heart failure. Biomarker profiling might add value to this approach, and a number have been assessed, with inflammatory markers being the most promising (Braunwald, 2008). Given the strong prospective data relating serum MK to patient survival in heart failure (Kitahara *et al.*, 2010), and MK's recognized role in inflammation (Weckbach *et al.*, 2011; Sakamoto and Kadomatsu, 2012), further assessment of MK both alone and in conjunction with other promising biomarkers may be a promising avenue of investigation.

MK biology: significant remaining questions

Although numerous studies have been published on the many facets of MK, a number of important fundamental questions about its basic structure, biology and bodily distribution remain unanswered. Addressing these questions will in turn help to understand further the significance of elevated MK levels.

MK variants

MK is a 13 kDa protein, produced from a single copy *Mdk* gene at chromosomal locus 11p11.2 (Kaname *et al.*, 1993). No glycosylation or other post-translational modifications have been reported for MK. The *Mdk* gene consists of five exons

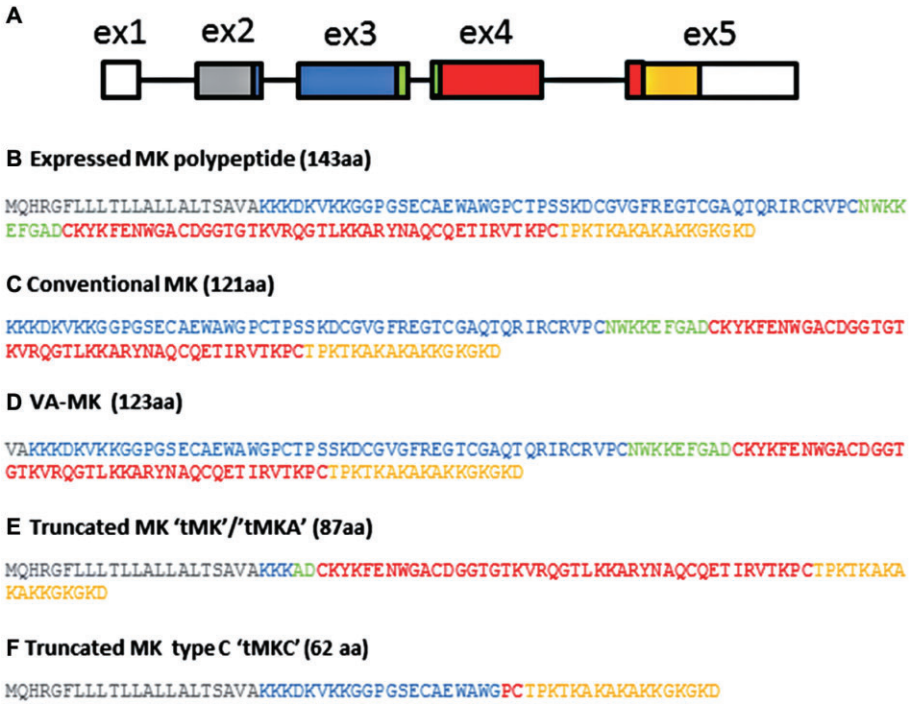

Figure 2

Known expressed variants of MK protein, adapted from Muramatsu (2011) and Tao *et al.* (2007). (A) Exon arrangement of the *Mdk* gene. Lines show introns; boxes show exons (ex1–5). Colours show location of the sequences coding for the MK signal peptide (grey), the N-terminus and N-domain (blue), the N-C linker sequence (green), the C-domain (red) and the N-terminus (orange). (B) Fully expressed polypeptide sequence of MK prior to signal peptide cleavage. Colours denote exo location according to (A). (C) 'Conventional MK' 121 amino acid sequence. (D) 'VA-MK' 123 amino acid sequence. (E) tMK ('tMK'/'tMKA') 87 amino acid sequence encoded by exon 3 skipping, resulting in deletion of the N-domain and most of the N-terminus. (F) tMK type c ('tMKC') 62 amino acid sequence, coded by skipping exon 4 and some of exon 3, resulting in deletion of most of the N-domain plus the C-domain.

(four coding and one non-coding; Figure 2A), which together express a 143 amino acid polypeptide in humans. This includes a signal sequence that is cleaved prior to secretion of the final MK protein (Uehara *et al.*, 1992).

The earliest report on the structure and sequence of (murine) *Mdk* demarked a putative signal sequence consisting of the first 22 amino acids at the N-terminus of the protein product (Matsubara *et al.*, 1990). When the human MDK gene was cloned and sequenced soon after, the same 22 amino acids were assumed to be the human putative signal sequence, hence giving a 121 amino acid human MK (Tsutsui *et al.*, 1991). Subsequently, most MK researchers have assumed that the biologically active form of MK is indeed the 121 amino acid species (Muramatsu, 1993), shown in Figure 2C ('Conventional-MK'). This assumption has remained the case ever since (Muramatsu, 2011; Sakamoto and Kadomatsu, 2012).

However, in the only published study where the MK protein itself has been isolated from human peripheral blood and analysed, N-terminal amino acid sequencing revealed valine and alanine as the first two MK residues on the recovered protein (Novotny *et al.*, 1993), revealing a MK species beginning two residues upstream in the signal sequence that yields a 123 amino acid variant ('VA-MK'; Figure 2D).

In Novotny *et al.*, the only MK isolate reported was VA-MK. However, using different methodologies, both conventional MK and VA-MK have been recovered from the same serum sample of a healthy volunteer (Muramatsu, 2014). Furthermore, a mixture of conventional and VA-MK proteins (at an approximate ratio of 35% MK: 65% VA-MK) was isolated from culture supernatants of the breast cancer cell line MCF-7 (Shoyab *et al.*, 1991). Therefore, it seems likely that both conventional and VA-MK may occur simultaneously *in vivo*. It is not known whether VA-MK has different functions or activities to those of conventional MK and further studies are clearly warranted. One aspect that the VA-N-terminus might affect is $t_{1/2}$ (see separate discussion of this issue below). Measuring VA-MK levels specifically and the differences in the relative levels of conventional MK and VA-MK in healthy and diseased subjects might reveal further diagnostic opportunities for MK. Capturing and measuring VA-MK would require highly specific reagents (monoclonal antibodies, aptamers) that differentiated VA-MK from conventional MK. The currently developed MK-specific reagents probably recognize both variants equally well. As such, the studies of circulating MK listed in Table 1 probably quantify VA-MK plus conventional MK.

As well as VA-MK, two truncated MK (tMK) variants have been reported in the literature, designated tMK (or tMKA in some later publications) and tMKC respectively (Figure 2E,F). tMK/tMKA results from an alternatively spliced mRNA that skips exon 3 (Kaname *et al.*, 1996) to give a protein of 87

amino acids. tMKC results from a deletion of part of exon 3 plus most of exon 4, encoding a putative 62 amino acid products (Tao *et al.*, 2007). Expression of tMKC is so far only evident at the gene level in 3 of out 4 human cancer cell lines and 2 out of 9 different resected cancer tissues tested (Tao *et al.*, 2007). Evidence of the expressed tMKC protein product has not yet been reported in any cell line or tissue.

tMK/tMKA gene expression has been identified in tumour, but not adjacent healthy, tissue in colorectal, pancreatic, hepatocellular, gastric, oesophageal and breast cancers (Miyashiro *et al.*, 1996; 1997; Aridome *et al.*, 1998). The tMK protein has also been identified in Wilms's tumour tissues (Paul *et al.*, 2001). Most strikingly, tMK/tMKA expression appears to be strongly correlated to lymph node metastasis; in a study of 10 patients with a variety of metastatic gastrointestinal cancers, 100% of the lymph node metastases (14 in total) were tMK mRNA positive (Aridome *et al.*, 1998). This apparent close association of tMK with metastasis is supported by *in vitro* functional studies in which cancer cells transfected with tMK more easily detached from fibronectin and were more invasive than those transfected with conventional MK (Akuzawa *et al.*, 2007).

Alternative splicing of genes has been widely reported for growth factors and their receptors (Tischer *et al.*, 1991; Kretschmer *et al.*, 1993; Marchionni *et al.*, 1993; Hattori *et al.*, 1996; Ornitz *et al.*, 1996), presumably as a mechanism whereby extra signalling complexity and diversity can evolve (Blencowe, 2006). Similarly, it may be that alternatively spliced MK species offer different functions to the single *Mdk* gene. Further studies of expression and function of variant MKs in health and disease are clearly warranted. Measuring the relative expression levels of full length versus variant MKs (both gene and protein, in tissues and in body fluids) might prove to be diagnostically useful, with early studies suggesting tMK may signify particularly aggressive and invasive cancer.

MK bio-distribution and $t_{1/2}$

Two fundamental aspects of MK that remain unknown are its biological $t_{1/2}$ and its bio-distribution. Both of these properties are pertinent to understanding MK measurements, particularly those taken from blood or other body fluids. These properties may also differ for different MK variants. For instance, VA-MK may well have a much longer *in vivo* $t_{1/2}$ than conventional MK, according to the N-end rule of protein stability (Gonda *et al.*, 1989).

Understanding the normal bio-distribution of MK will also enhance understanding of the significance of circulating MK levels. Bio-distribution is most probably intrinsically related to $t_{1/2}$ and clearance. Although formal, whole-body studies of MK bio-distribution *per se* have not been conducted, data from several other studies provide some relevant insights. Circulating MK levels are rapidly and massively elevated when healthy human subjects are injected i.v. with heparin (Fujisawa *et al.*, 1998; Hung *et al.*, 2011). Both of these studies found that within 30 min of heparin injection, serum MK levels went as high as 350 $ng{\cdot}mL^{-1}$ (depending on the heparin dose), or approximately 1000 times the expected normal healthy circulating MK levels (Figure 1). Peak MK levels following heparin injection were reached 15–30 min after injection, which suggests that pre-existing MK was released into the circulation, as opposed to heparin stimulating *de novo* MK expression and synthesis. For this to be the case, a large pool of immobilized MK must be sequestered somewhere in the body that is easily accessible to quick release by circulating heparin. The most obvious and likely site of sequestration is the internal surface of the vasculature itself (Kadomatsu *et al.*, 2013). MK binds to a variety of sulphated glycosaminoglycans in proteoglycans (Muramatsu, 2010), with affinities (K_D) typically in the mid- to high-nM range (Sugiura *et al.*, 2012). A number of proteoglycans have been identified as putative 'MK receptors', including syndecans-1 and -3 and protein tyrosine phosphatase ζ. Because MK is a heparin-binding protein, heparan sulphate proteoglycans (HSPGs) such as the syndecans and integrins are prime candidates for the MK-sequestering receptors. Indeed MK binds to syndecans (Mitsiadis *et al.*, 1995; Kojima *et al.*, 1996; Nakanishi *et al.*, 1997) and some integrins (Muramatsu *et al.*, 2004). HSPGs are critical modulators of growth factor activities, and the sequestering of growth factors at endothelial surfaces by HSPGs is a widely observed feature of growth factor biology (Gotte, 2003; Rusnati and Presta, 2006). Because MK has a low nM affinity (K_D) for heparin (Kadomatsu, 2008), injection of soluble heparin is likely to displace MK from, endothelial proteoglycans to release MK–heparin complexes into the circulation.

A corollary of this is that MK secreted during disease should in theory be sequestered rapidly by the vascular bed and it seems reasonable to postulate that the entire circulatory system of a patient has a relatively large capacity to absorb newly synthesized MK. A fruitful area of future MK research may be to discern whether the MK being expressed by, for example, a growing tumour is initially 'mopped up' into this sink for some time before it becomes evident as elevated blood MK, or whether some property of the tumour MK ensures that it stays in circulation upon secretion, making it detectable immediately as elevated blood MK.

Conclusion

MK has already proved its utility as a biomarker in several clinical oncology settings, and a large body of literature strongly suggests that it would be useful in a number of other cancers. Opportunities to employ MK as a cancer biomarker exist throughout the disease history, from initial population screening to monitoring for recurrence. Depending on the particular clinical circumstance, measuring MK alone or in combination with other biomarkers offers utility. MK also shows early promise as a biomarker in non-oncological conditions, but further studies are needed. Future studies of MK as a biomarker in both cancer and non-cancer diseases should focus on showing utility in addressing specific clinical problems; they should be prospective where possible, or blinded 'retrospective–prospective' studies; and MK should be trialled against the current best practice test in the same clinical setting. Finally, significant opportunities in developing novel clinical tests lie in further investigating the occurrence of tMK and other MK variants, in relation to disease.

Conflict of interest

D. R. J. is employed by Cellmid Ltd., a biotechnology company that is commercializing MK-based diagnostics and therapeutics.

References

Aberle DR, Adams AM, Berg CD, Black WC, Clapp JD, Fagerstrom RM *et al.* (2011). Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 365: 395–409.

Abogunrin F, O'Kane HF, Ruddock MW, Stevenson M, Reid CN, O'Sullivan JM *et al.* (2011). The impact of biomarkers in multivariate algorithms for bladder cancer diagnosis in patients with hematuria. Cancer 118: 2641–2650.

Akuzawa N, Nobata S, Shinozawa T (2007). Truncated midkine correlates with sensitivity to anticancer drugs and malignancy in human tumor cell line. Cell Biochem Funct 25: 687–691.

Aridome K, Tsutsui J, Takao S, Kadomatsu K, Ozawa M, Aikou T *et al.* (1995). Increased midkine gene expression in human gastrointestinal cancers. Jpn J Cancer Res 86: 655–661.

Aridome K, Takao S, Kaname T, Kadomatsu K, Natsugoe S, Kijima F *et al.* (1998). Truncated midkine as a marker of diagnosis and detection of nodal metastases in gastrointestinal carcinomas. Br J Cancer 78: 472–477.

Birse C, Lagier RJ, Bruce RN, Tomic JL, Ruben SM, Lenk TJ *et al.* (2011). Protein biomarker panel accurately resolves non-malignant nodules identified through low-dose helical CT scanning. In: 14th World Conference on Lung Cancer, Session Info: Oral Session OBI, Day/Date: Tuesday J, 2011, PM STA-, E106 R, | AO (eds). Amsterdam.

Blencowe BJ (2006). Alternative splicing: new insights from global analyses. Cell 126: 37–47.

Boja ES, Jortani SA, Ritchie J, Hoofnagle AN, Tezak Z, Mansfield E *et al.* (2011). The journey to regulation of protein-based multiplex quantitative assays. Clin Chem 57: 560–567.

Braunwald E (2008). Biomarkers in heart failure. N Engl J Med 358: 2148–2159.

Cellmid (2013). Midkine ELISA kit: instructions for use.

Centola M, Cavet G, Shen Y, Ramanujan S, Knowlton N, Swan KA *et al.* (2013). Development of a multi-biomarker disease activity test for rheumatoid arthritis. PLoS ONE 8: e60635.

Cohen S, Shoshana O, Zelman-Toister E, Maharshak N, Binsky-Ehrenreich I, Gordin M *et al.* (2011). The cytokine midkine and its receptor RPTPζ regulate B cell survival in a pathway induced by CD74. J Immunol 188: 259–269.

Devarajan P (2011). Biomarkers for the early detection of acute kidney injury. Curr Opin Pediatr 23: 194–200.

Fiala R, Stejskal D, Vidlar A, Grepl M (2006). Evaluation of 5 potential biomarkers for urinary bladder cancer – endostatin, midkine, survivin, livin and E-FABP. Eur Urol Suppl 5: 253.

Fiegel HC, Kaifi JT, Wachowiak R, Quaas A, Aridome K, Ichihara-Tanaka K *et al.* (2008). Midkine is highly expressed in neuroblastoma tissues. Pediatr Surg Int 24: 1355–1359.

Fujisawa K, Matsumoto Y, Muramatsu H, Shinzato T, Hiramatsu K, Horie K *et al.* (1998). Increased serum midkine levels during hemodialysis using heparin in chronic renal failure. J Biochem 123: 864–869.

Fukui S, Kitagawa-Sakakida S, Kawamata S, Matsumiya G, Kawaguchi N, Matsuura N *et al.* (2008). Therapeutic effect of midkine on cardiac remodeling in infarcted rat hearts. Ann Thorac Surg 85: 562–570.

Garver RI Jr, Chan CS, Milner PG (1993). Reciprocal expression of pleiotrophin and midkine in normal versus malignant lung tissues. Am J Respir Cell Mol Biol 9: 463–466.

Gonda DK, Bachmair A, Wünning I, Tobias JW, Lane WS, Varshavsky A (1989). Universality and structure of the N-end rule. J Biol Chem 264: 16700–16712.

Gotte M (2003). Syndecans in inflammation. FASEB J 17: 575–591.

Han WK, Waikar SS, Johnson A, Betensky RA, Dent CL, Devarajan P *et al.* (2008). Urinary biomarkers in the early diagnosis of acute kidney injury. Kidney Int 73: 863–869.

Hattori Y, Yamasaki M, Itoh N (1996). The rat FGF-5 mRNA variant generated by alternative splicing encodes a novel truncated form of FGF-5. Biochim Biophys Acta 1306: 31–33.

Hayashi H, Sato W, Maruyama S, Sugiyama D, Ikematsu S, Nishiwaki K *et al.* (2009). Urinary midkine as a biomarker of acute kidney injury: comparison with three major biomarkers; NAG, IL-18 and NGAL 24 In: World Congress of Nephrology Vol. 2, p ii1634. Milan.

Hayes DF, Allen J, Compton C, Gustavsen G, Leonard DGB, McCormack R *et al.* (2013). Breaking a vicious cycle. Sci Transl Med 5: 196cm196.

Heidenreich A, Bellmunt J, Bolla M, Joniau S, Mason M, Matveev V *et al.* (2011). EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and treatment of clinically localised disease. Eur Urol 59: 61–71.

Heidenreich A, Abrahamsson PA, Artibani W, Catto J, Montorsi F, Van Poppel H *et al.* (2013). Early detection of prostate cancer: European association of urology recommendation. Eur Urol 64: 347–354.

Horiba M, Kadomatsu K, Nakamura E, Muramatsu H, Ikematsu S, Sakuma S *et al.* (2000). Neointima formation in a restenosis model is suppressed in midkine-deficient mice. J Clin Invest 105: 489–495.

Horiba M, Kadomatsu K, Yasui K, Lee JK, Takenaka H, Sumida A *et al.* (2006). Midkine plays a protective role against cardiac ischemia/reperfusion injury through a reduction of apoptotic reaction. Circulation 114: 1713–1720.

Huang Y, Cao G, Wang H, Wang Q, Hou Y (2007). The expression and location of midkine in gastric carcinomas of Chinese patients. Cell Mol Immunol 4: 135–140.

Hung Y-J, Lin ZHY, Cheng T-I, Liang C-T, Kuo T-M, Kao K-J (2011). Serum midkine as a prognostic biomarker for patients with hepatocellular carcinoma. Am J Clin Pathol 136: 594–603.

Ibusuki M, Fujimori H, Yamamoto Y, Ota K, Ueda M, Shinriki S *et al.* (2009). Midkine in plasma as a novel breast cancer marker. Cancer Sci 100: 1735–1739.

Ikematsu S, Yano A, Aridome K, Kikuchi M, Kumai H, Nagano H *et al.* (2000). Serum midkine levels are increased in patients with various types of carcinomas. Br J Cancer 83: 701–706.

Ikematsu S, Okamoto K, Yoshida Y, Oda M, Sugano-Nagano H, Ashida K *et al.* (2003). High levels of urinary midkine in various cancer patients. Biochem Biophys Res Commun 306: 329–332.

Ikematsu S, Nakagawara A, Nakamura Y, Ohira M, Shinjo M, Kishida S *et al.* (2008). Plasma midkine level is a prognostic factor for human neuroblastoma. Cancer Sci 99: 2070–2074.

Ilic D, Neuberger MM, Djulbegovic M, Dahm P (2013). Screening for prostate cancer. Cochrane Database Syst Rev (1): CD004720.

Ishiguro H, Horiba M, Takenaka H, Sumida A, Opthof T, Ishiguro YS *et al*. (2011). A single intracoronary injection of midkine reduces ischemia/reperfusion injury in swine hearts: a novel therapeutic approach for acute coronary syndrome. Front Physiol 2: 27.

Ishikawa E, Ooboshi H, Kumai Y, Takada J, Nakamura K, Ago T *et al*. (2009). Midkine gene transfer protects against focal brain ischemia and augments neurogenesis. J Neurol Sci 285: 78–84.

Jham BC, Costa NL, Silva JM, de Miranda AC, Oliveira JC, Silva TA *et al*. (2011). Midkine expression in oral squamous cell carcinoma and leukoplakia. J Oral Pathol Med 41: 21–26.

Jia HL, Ye QH, Qin LX, Budhu A, Forgues M, Chen Y *et al*. (2007). Gene expression profiling reveals potential biomarkers of human hepatocellular carcinoma. Clin Cancer Res 13: 1133–1139.

Jones HB (1848). On a new substance occurring in the urine of a patient with mollities ossium. Philos Trans R Soc Lond 138: 55–62.

Kadomatsu K (2008) The midkine family and its receptors. In: Taniguchi N, Suzuki A, Ito Y, Narimatsu H, Kawasaki T, Hase S (eds). Experimental Glycoscience. Springer: London, pp. 355–357.

Kadomatsu K, Tomomura M, Muramatsu T (1988). cDNA cloning and sequencing of a new gene intensely expressed in early differentiation stages of embryonal carcinoma cells and in mid-gestation period of mouse embryogenesis. Biochem Biophys Res Commun 151: 1312–1318.

Kadomatsu K, Huang RP, Suganuma T, Murata F, Muramatsu T (1990). A retinoic acid responsive gene MK found in the teratocarcinoma system is expressed in spatially and temporally controlled manner during mouse embryogenesis. J Cell Biol 110: 607–616.

Kadomatsu K, Kishida S, Tsubota S (2013). The heparin-binding growth factor midkine: the biological activities and candidate receptors. J Biochem 153: 511–521.

Kaifi JT, Fiegel HC, Rafnsdottir SL, Aridome K, Schurr PG, Reichelt U *et al*. (2007). Midkine as a prognostic marker for gastrointestinal stromal tumors. J Cancer Res Clin Oncol 133: 431–435.

Kaname T, Kuwano A, Murano I, Uehara K, Muramatsu T, Kajii T (1993). Midkine gene (MDK), a gene for prenatal differentiation and neuroregulation, maps to band 11p11.2 by fluorescence *in situ* hybridization. Genomics 17: 514–515.

Kaname T, Kadomatsu K, Aridome K, Yamashita S, Sakamoto K, Ogawa M *et al*. (1996). The expression of truncated MK in human tumors. Biochem Biophys Res Commun 219: 256–260.

Kato K, Kosugi T, Sato W, Arata-Kawai H, Ozaki T, Tsuboi N *et al*. (2011). Growth factor Midkine is involved in the pathogenesis of renal injury induced by protein overload containing endotoxin. Clin Exp Nephrol 15: 346–364.

Kawai H, Sato W, Yuzawa Y, Kosugi T, Matsuo S, Takei Y *et al*. (2004). Lack of the growth factor midkine enhances survival against cisplatin-induced renal damage. Am J Pathol 165: 1603–1612.

Kemik O, Sumer A, Kemik AS, Hasirci I, Purisa S, Dulger AC *et al*. (2010). The relationship among acute-phase response proteins, cytokines and hormones in cachectic patients with colon cancer. World J Surg Oncol 8: 85.

Kitahara T, Shishido T, Suzuki S, Katoh S, Sasaki T, Ishino M *et al*. (2010). Serum midkine as a predictor of cardiac events in patients with chronic heart failure. J Card Fail 16: 308–313.

Koide N, Hada H, Shinji T, Ujike K, Hirasaki S, Yumoto Y *et al*. (1999). Expression of the midkine gene in human hepatocellular carcinomas. Hepatogastroenterology 46: 3189–3196.

Kojima T, Katsumi A, Yamazaki T, Muramatsu T, Nagasaka T, Ohsumi K *et al*. (1996). Human ryudocan from endothelium-like cells binds basic fibroblast growth factor, midkine, and tissue factor pathway inhibitor. J Biol Chem 271: 5914–5920.

Konety BR (2006). Molecular markers in bladder cancer: a critical appraisal. Urol Oncol 24: 326–337.

Konishi N, Nakamura M, Nakaoka S, Hiasa Y, Cho M, Uemura H *et al*. (1999). Immunohistochemical analysis of midkine expression in human prostate carcinoma. Oncology 57: 253–257.

Kosugi T, Sato W (2012). Midkine and the kidney: health and diseases. Nephrol Dial Transplant 27: 16–21.

Kosugi T, Yuzawa Y, Sato W, Arata-Kawai H, Suzuki N, Kato N *et al*. (2007). Midkine is involved in tubulointerstitial inflammation associated with diabetic nephropathy. Lab Invest 87: 903–913.

Kretschmer PJ, Fairhurst JL, Hulmes JD, Popjes ML, Bohlen P, Kovesdi I (1993). Genomic organization of the human HBNF gene and characterization of an HBNF variant protein as a splice mutant. Biochem Biophys Res Commun 192: 420–429.

Krzystek-Korpacka M, Matusiewicz M (2012) Circulating midkine in malignant, inflammatory, and infectious diseases: a systematic review. In: Ergüven M, Muramatsu T, Bilir A (eds). Midkine: From Embryogenesis to Pathogenesis and Therapy. Springer: London, pp. 69–85.

Krzystek-Korpacka M, Matusiewicz M, Diakowska D, Grabowski K, Blachut K, Kustrzeba-Wojcicka I *et al*. (2007). Serum midkine depends on lymph node involvement and correlates with circulating VEGF-C in oesophageal squamous cell carcinoma. Biomarkers 12: 403–413.

Krzystek-Korpacka M, Neubauer K, Matusiewicz M (2010). Circulating midkine in Crohn's disease: clinical implications. Inflamm Bowel Dis 16: 208–215.

Krzystek-Korpacka M, Mierzchala M, Neubauer K, Durek G, Gamian A (2011). Midkine, a multifunctional cytokine, in patients with severe sepsis and septic shock: a pilot study. Shock 35: 471–477.

Krzystek-Korpacka M, Diakowska D, Neubauer K, Gamian A (2013). Circulating midkine in malignant and non-malignant colorectal diseases. Cytokine 64: 158–164.

Lee YJ, Park JE, Jeon BR, Lee SM, Kim SY, Lee YK (2013). Is prostate-specific antigen effective for population screening of prostate cancer? A systematic review. Ann Lab Med 33: 233–241.

Lin K, Croswell JM, Koenig H, Lam C, Maltz A (2011). Prostate-specific antigen-based screening for prostate cancer: an evidence update for the U.S. preventive services task force [Internet], 2011/12/16 edn. Available at: http://www.ncbi.nlm.nis.gov/pubmedhealth/PMH0032900/ (accessed 3/9/2013).

Ling MM, Ricks C, Lea P (2007). Multiplexing molecular diagnostics and immunoassays using emerging microarray technologies. Expert Rev Mol Diagn 7: 87–98.

Liu X, Mashour GA, Webster HF, Kurtz A (1998). Basic FGF and FGF receptor 1 are expressed in microglia during experimental autoimmune encephalomyelitis: temporally distinct expression of midkine and pleiotrophin. Glia 24: 390–397.

Loeb S, Vonesh EF, Metter EJ, Carter HB, Gann PH, Catalona WJ (2011). What is the true number needed to screen and treat to save a life with prostate-specific antigen testing? J Clin Oncol 29: 464–467.

Lucas S, Reindl T, Henze G, Kurtz A, Sakuma S, Driever PH (2009). Increased midkine serum levels in pediatric embryonal tumor patients. J Pediatr Hematol Oncol 31: 713–717.

Luo Q, Zhou F, Dong H, Wu L, Chai L, Lan K *et al*. (2013). Implication of combined urinary biomarkers in early diagnosis of acute kidney injury following percutaneous coronary intervention. Clin Nephrol 79: 85–92.

Maeda S, Shinchi H, Kurahara H, Mataki Y, Noma H, Maemura K *et al*. (2007). Clinical significance of midkine expression in pancreatic head carcinoma. Br J Cancer 97: 405–411.

Maehara H, Kaname T, Yanagi K, Hanzawa H, Owan I, Kinjou T *et al*. (2007). Midkine as a novel target for antibody therapy in osteosarcoma. Biochem Biophys Res Commun 358: 757–762.

Mamtani MR, Thakre TP, Kalkonde MY, Amin MA, Kalkonde YV, Amin AP *et al*. (2006). A simple method to combine multiple molecular biomarkers for dichotomous diagnostic classification. BMC Bioinformatics 7: 442.

Marchionni MA, Goodearl AD, Chen MS, Bermingham-McDonogh O, Kirk C, Hendricks M *et al*. (1993). Glial growth factors are alternatively spliced erbB2 ligands expressed in the nervous system. Nature 362: 312–318.

Maruyama K, Muramatsu H, Ishiguro N, Muramatsu T (2004). Midkine, a heparin-binding growth factor, is fundamentally involved in the pathogenesis of rheumatoid arthritis. Arthritis Rheum 50: 1420–1429.

Mashour GA, Wang HL, Cabal-Manzano R, Wellstein A, Martuza RL, Kurtz A (1999). Aberrant cutaneous expression of the angiogenic factor midkine is associated with neurofibromatosis type-1. J Invest Dermatol 113: 398–402.

Mashour GA, Driever PH, Hartmann M, Drissel SN, Zhang T, Scharf B *et al*. (2004). Circulating growth factor levels are associated with tumorigenesis in neurofibromatosis type 1. Clin Cancer Res 10: 5677–5683.

Matsubara S, Tomomura M, Kadomatsu K, Muramatsu T (1990). Structure of a retinoic acid-responsive gene, MK, which is transiently activated during the differentiation of embryonal carcinoma cells and the mid-gestation period of mouse embryogenesis. J Biol Chem 265: 9441–9443.

Mishima K, Asai A, Kadomatsu K, Ino Y, Nomura K, Narita Y *et al*. (1997). Increased expression of midkine during the progression of human astrocytomas. Neurosci Lett 233: 29–32.

Mitsiadis TA, Muramatsu T, Muramatsu H, Thesleff I (1995). Midkine (MK), a heparin-binding growth/differentiation factor, is regulated by retinoic acid and epithelial–mesenchymal interactions in the developing mouse tooth, and affects cell proliferation and morphogenesis. J Cell Biol 129: 267–281.

Miyashiro I, Kaname T, Nakayama T, Nakamori S, Yagyu T, Monden T *et al*. (1996). Expression of truncated midkine in human colorectal cancers. Cancer Lett 106: 287–291.

Miyashiro I, Kaname T, Shin E, Wakasugi E, Monden T, Takatsuka Y *et al*. (1997). Midkine expression in human breast cancers: expression of truncated form. Breast Cancer Res Treat 43: 1–6.

Mochizuki R, Takeda A, Sato N, Kimpara T, Onodera H, Itoyama Y *et al*. (1998). Induction of midkine expression in reactive astrocytes following rat transient forebrain ischemia. Exp Neurol 149: 73–78.

Moon HS, Park WI, Sung SH, Choi EA, Chung HW, Woo BH (2003). Immunohistochemical and quantitative competitive PCR analyses of midkine and pleiotrophin expression in cervical cancer. Gynecol Oncol 88: 289–297.

Moyer VA (2012). Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 157: 120–134.

Muramatsu H, Shirahama H, Yonezawa S, Maruta H, Muramatsu T (1993). Midkine, a retinoic acid-inducible growth/differentiation factor: immunochemical evidence for the function and distribution. Dev Biol 159: 392–402.

Muramatsu H, Zou P, Suzuki H, Oda Y, Chen GY, Sakaguchi N *et al*. (2004). alpha4beta1- and alpha6beta1-integrins are functional receptors for midkine, a heparin-binding growth factor. J Cell Sci 117: 5405–5415.

Muramatsu T (1993). Midkine (MK), the product of a retinoic acid responsive gene, and pleiotrophin constitute a new protein family regulating growth and differentiation. Int J Dev Biol 37: 183–188.

Muramatsu T (2010). Midkine, a heparin-binding cytokine with multiple roles in development, repair and diseases. Proc Jpn Acad Ser B Phys Biol Sci 86: 410–425.

Muramatsu T (2011). Midkine: a promising molecule for drug development to treat diseases of the central nervous system. Curr Pharm Des 17: 410–423.

Muramatsu T (2014). Structure and function of midkine as the basis of its pharmacological effects. Br J Pharmacol 171: 814–826.

Nakagawara A, Milbrandt J, Muramatsu T, Deuel TF, Zhao H, Cnaan A *et al*. (1995). Differential expression of pleiotrophin and midkine in advanced neuroblastomas. Cancer Res 55: 1792–1797.

Nakamoto M, Matsubara S, Miyauchi T, Obama H, Ozawa M, Muramatsu T (1992). A new family of heparin binding growth/differentiation factors: differential expression of the midkine (MK) and HB-GAM genes during mouse development. J Biochem 112: 346–349.

Nakanishi T, Kadomatsu K, Okamoto T, Tomoda Y, Muramatsu T (1997). Expression of midkine and pleiotropin in ovarian tumors. Obstet Gynecol 90: 285–290.

Nordin A, Wang W, Welen K, Damber JE (2013). Midkine is associated with neuroendocrine differentiation in castration-resistant prostate cancer. Prostate 73: 657–667.

Novotny WF, Maffi T, Mehta RL, Milner PG (1993). Identification of novel heparin-releasable proteins, as well as the cytokines midkine and pleiotrophin, in human postheparin plasma. Arterioscler Thromb 13: 1798–1805.

Obata Y, Kikuchi S, Lin Y, Yagyu K, Muramatsu T, Kumai H (2005). Serum midkine concentrations and gastric cancer. Cancer Sci 96: 54–56.

O'Brien T, Cranston D, Fuggle S, Bicknell R, Harris AL (1996). The angiogenic factor midkine is expressed in bladder cancer, and overexpression correlates with a poor outcome in patients with invasive cancers. Cancer Res 56: 2515–2518.

Ohhashi S, Ohuchida K, Mizumoto K, Egami T, Yu J, Cui L *et al*. (2009). Midkine mRNA is overexpressed in pancreatic cancer. Dig Dis Sci 54: 811–815.

Ornitz DM, Xu J, Colvin JS, McEwen DG, MacArthur CA, Coulier F *et al*. (1996). Receptor specificity of the fibroblast growth factor family. J Biol Chem 271: 15292–15297.

Ostroff RM, Mehan MR, Stewart A, Ayers D, Brody EN, Williams SA *et al*. (2012). Early detection of malignant pleural mesothelioma in asbestos-exposed individuals with a noninvasive proteomics-based surveillance tool. PLoS ONE 7: e46091.

O'Sullivan P, Sharples K, Dalphin M, Davidson P, Gilling P, Cambridge L *et al*. (2012). A multigene urine test for the detection and stratification of bladder cancer in patients presenting with hematuria. J Urol 188: 741–747.

Ota K, Fujimori H, Ueda M, Shiniriki S, Kudo M, Jono H *et al.* (2008). Midkine as a prognostic biomarker in oral squamous cell carcinoma. Br J Cancer 99: 655–662.

Ota K, Fujimori H, Ueda M, Jono H, Shinriki S, Ota T *et al.* (2010). Midkine expression is correlated with an adverse prognosis and is down-regulated by p53 in oral squamous cell carcinoma. Int J Oncol 37: 797–804.

Paul S, Mitsumoto T, Asano Y, Kato S, Kato M, Shinozawa T (2001). Detection of truncated midkine in Wilms' tumor by a monoclonal antibody against human recombinant truncated midkine. Cancer Lett 163: 245–251.

Rawnaq T, Kunkel M, Bachmann K, Simon R, Zander H, Brandl S *et al.* (2011). Serum midkine correlates with tumor progression and imatinib response in gastrointestinal stromal tumors. Ann Surg Oncol 18: 559–565.

Rha SY, Noh SH, Kwak HJ, Wellstein A, Kim JH, Roh JK *et al.* (1997). Comparison of biological phenotypes according to midkine expression in gastric cancer cells and their autocrine activities could be modulated by pentosan polysulfate. Cancer Lett 118: 37–46.

Rice GE, Edgell TA, Autelitano DJ (2010). Evaluation of midkine and anterior gradient 2 in a multimarker panel for the detection of ovarian cancer. J Exp Clin Cancer Res 29: 62.

Ruan M, Ji T, Wu Z, Zhou J, Zhang C (2007). Evaluation of expression of midkine in oral squamous cell carcinoma and its correlation with tumour angiogenesis. Int J Oral Maxillofac Surg 36: 159–164.

Rusnati M, Presta M (2006). Extracellular angiogenic growth factor interactions: an angiogenesis interactome survey. Endothelium 13: 93–111.

Sakamoto K, Kadomatsu K (2012). Midkine in the pathology of cancer, neural disease, and inflammation. Pathol Int 62: 445–455.

Salama RH, Muramatsu H, Shimizu E, Hashimoto K, Ohgake S, Watanabe H *et al.* (2005). Increased midkine levels in sera from patients with Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 29: 611–616.

Salaru DL, Mertens PR, Bartsch P (2013). Loss of heparin-binding protein prevents necrotizing glomerulonephritis: first clues hint at plasminogen activator inhibitor-1. Int Urol Nephrol 45: 1483–1487.

Sato W, Kadomatsu K, Yuzawa Y, Muramatsu H, Hotta N, Matsuo S *et al.* (2001). Midkine is involved in neutrophil infiltration into the tubulointerstitium in ischemic renal injury. J Immunol 167: 3463–3469.

Satoh J, Muramatsu H, Moretto G, Muramatsu T, Chang HJ, Kim ST *et al.* (1993). Midkine that promotes survival of fetal human neurons is produced by fetal human astrocytes in culture. Brain Res Dev Brain Res 75: 201–205.

Scheerer J, Kahabka P, Altwein JE, Weissbach L (2012). Unusually large numbers needed to treat for radical prostatectomy in prostate cancer patients with PSA velocity ≤2 ng/mL/year. Urol Int 89: 155–161.

Shimada H, Nabeya Y, Tagawa M, Okazumi S, Matsubara H, Kadomatsu K *et al.* (2003). Preoperative serum midkine concentration is a prognostic marker for esophageal squamous cell carcinoma. Cancer Sci 94: 628–632.

Shoyab M, McDonald VL, Dick K, Modrell B, Malik N, Plowman GD (1991). Amphiregulin-associated protein: complete amino acid sequence of a protein produced by the 12-0-tetradecanoylphorbol-13-acetate-treated human breast adenocarcinoma cell line MCF-7. Biochem Biophys Res Commun 179: 572–578.

Sommerschuh S, Kirchner M, Gliese N (2012) Immunochemical methods for the detection of midkine in samples of human origin. In: Ergüven M, Muramatsu T, Bilir A (eds). Midkine: From Embryogenesis to Pathogenesis and Therapy. Springer: London, pp. 53–67.

Sonobe Y, Li H, Jin S, Kishida S, Kadomatsu K, Takeuchi H *et al.* (2012). Midkine inhibits inducible regulatory T cell differentiation by suppressing the development of tolerogenic dendritic cells. J Immunol 188: 2602–2611.

Sorbye H, Mauer M, Gruenberger T, Glimelius B, Poston GJ, Schlag PM *et al.* (2012). Predictive factors for the benefit of perioperative FOLFOX for resectable liver metastasis in colorectal cancer patients (EORTC Intergroup Trial 40983). Ann Surg 255: 534–539.

Soulie P, Heroult M, Bernard-Pierrot I, Caruelle D, Oglobine J, Barritault D *et al.* (2004). Correlation of elevated plasma levels of two structurally related growth factors, heparin affin regulatory peptide and midkine, in advanced solid tumor patients. Cancer Detect Prev 28: 319–324.

Stylianou DC, Auf der Maur A, Kodack DP, Henke RT, Hohn S, Toretsky JA *et al.* (2009). Effect of single-chain antibody targeting of the ligand-binding domain in the anaplastic lymphoma kinase receptor. Oncogene 28: 3296–3306.

Su BB, Shi H, Wan J (2012). Role of serum carcinoembryonic antigen in the detection of colorectal cancer before and after surgical resection. World J Gastroenterol 18: 2121–2126.

Sueyoshi T, Jono H, Shinriki S, Ota K, Ota T, Tasaki M *et al.* (2011). Therapeutic approaches targeting midkine suppress tumor growth and lung metastasis in osteosarcoma. Cancer Lett 316: 23–30.

Sugiura N, Shioiri T, Chiba M, Sato T, Narimatsu H, Kimata K *et al.* (2012). Construction of a chondroitin sulfate library with defined structures and analysis of molecular interactions. J Biol Chem 287: 43390–43400.

Takada T, Toriyama K, Muramatsu H, Song XJ, Torii S, Muramatsu T (1997). Midkine, a retinoic acid-inducible heparin-binding cytokine in inflammatory responses: chemotactic activity to neutrophils and association with inflammatory synovitis. J Biochem 122: 453–458.

Takenaka H, Horiba M, Ishiguro H, Sumida A, Hojo M, Usui A *et al.* (2009). Midkine prevents ventricular remodeling and improves long-term survival after myocardial infarction. Am J Physiol Heart Circ Physiol 296: H462–H469.

Tao P, Xu D, Lin S, Ouyang GL, Chang Y, Chen Q *et al.* (2007). Abnormal expression, highly efficient detection and novel truncations of midkine in human tumors, cancers and cell lines. Cancer Lett 253: 60–67.

Tischer E, Mitchell R, Hartman T, Silva M, Gospodarowicz D, Fiddes JC *et al.* (1991). The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. J Biol Chem 266: 11947–11954.

Tomic JL, Bruce RJ, Lagier RJ, Birse CE (2012) Development of a multiplex test for detection of non small cell lung cancer (NSCLC). In: American Association for Clinical Chemistry Annual Meeting, Vol. 58. Clinical Chemistry, pp. A96–A97.

Tong Y, Mentlein R, Buhl R, Hugo HH, Krause J, Mehdorn HM *et al.* (2007). Overexpression of midkine contributes to anti-apoptotic effects in human meningiomas. J Neurochem 100: 1097–1107.

Tsutsui J, Uehara K, Kadomatsu K, Matsubara S, Muramatsu T (1991). A new family of heparin-binding factors: strong conservation of midkine (MK) sequences between the human and the mouse. Biochem Biophys Res Commun 176: 792–797.

Tsutsui J, Kadomatsu K, Matsubara S, Nakagawara A, Hamanoue M, Takao S *et al*. (1993). A new family of heparin-binding growth/differentiation factors: increased midkine expression in Wilms' tumor and other human carcinomas. Cancer Res 53: 1281–1285.

Uehara K, Matsubara S, Kadomatsu K, Tsutsui J, Muramatsu T (1992). Genomic structure of human midkine (MK), a retinoic acid-responsive growth/differentiation factor. J Biochem 111: 563–567.

Wang J, Takeuchi H, Sonobe Y, Jin S, Mizuno T, Miyakawa S *et al*. (2008). Inhibition of midkine alleviates experimental autoimmune encephalomyelitis through the expansion of regulatory T cell population. Proc Natl Acad Sci U S A 105: 3915–3920.

Weckbach LT, Muramatsu T, Walzog B (2011). Midkine in inflammation. ScientificWorldJournal 11: 2491–2505.

Weckbach LT, Groesser L, Borgolte J, Pagel J-I, Pogoda F, Schymeinsky J *et al*. (2012). Midkine acts as proangiogenic cytokine in hypoxia-induced angiogenesis. Am J Physiol Heart Circ Physiol 303: H429–H438.

Ye C, Qi M, Fan QW, Ito K, Akiyama S, Kasai Y *et al*. (1999). Expression of midkine in the early stage of carcinogenesis in human colorectal cancer. Br J Cancer 79: 179–184.

Yoshida Y, Goto M, Tsutsui J, Ozawa M, Sato E, Osame M *et al*. (1995). Midkine is present in the early stage of cerebral infarct. Brain Res Dev Brain Res 85: 25–30.

Zhao ZQ, Yang S, Lu HS (2012). Expression of midkine and vascular endothelial growth factor in gastric cancer and the association of high levels with poor prognosis and survival. Mol Med Rep 5: 415–419.

Zhu W-W, Guo J, Guo L, Jia H, Zhu M, Zhang J-B *et al*. (2013). Evaluation of midkine as a diagnostic serum biomarker in hepatocellular carcinoma. Clin Cancer Res 19: 3944–3954.