EDITORIAL

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Do matrix metalloproteinases represent reliable circulating biomarkers in colorectal cancer?

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Colorectal cancer (CRC) is one of the leading causes of worldwide cancer mortality. It accounts for almost 8% of all cancer deaths. Despite the significant advances in diagnosis, screening and treatment, there are limited therapeutic options for patients with advanced disease, highlighting the need for additional tumour molecular markers, circulating biomarkers and prognostic predictors (Liu *et al*, 2014; Lam *et al*, 2016; Lech *et al*, 2016; Yiu and Yiu, 2016).

In this respect, Matrix Metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) have been widely recognised playing a prominent role in cancer cells (Hadler-Olsen *et al*, 2013), especially in the processes of tumour invasion, progression and metastasis of CRC (Said *et al*, 2014).

The MMP family is represented by zinc-dependent endopeptidases belonging to the *Metzincin* superfamily, characterised by diverse structural domain architecture and different substrate specificity, and temporal and tissue specific expression pattern. MMPs are involved in crucial physiologic and pathologic processes, including tissue remodeling, stem cell differentiation and proliferation, apoptosis, as well as inflammation, tissue degeneration and cancer. The MMP family comprises different classes of proteinases able to degrade and/or activate proteins, that play crucial roles in tumour initiation, progression and metastases, suggesting them as possible diagnostic, prognostic, and therapeutic targets for cancer patients. Nevertheless, up to now it has been difficult to recognise MMPs as reliable CRC biomarkers and very complex to find specific MMP inhibitors as efficient therapeutic agents. In fact, previous clinical trials using MMP inhibitors were disappointing because off-target toxicity and a lack of efficacy (Said *et al*, 2014; Shai *et al*, 2015).

Because of the changes at the cellular level may be reflected in bodily fluids, increased concentrations and peculiar isoforms of MMPs in human blood may represent interesting and non-invasive laboratory tools for diagnosing and screening patients at higher risk for most cancer types (including bladder, brain, breast, lung, ovarian, pancreatic, prostate and colorectal tumour s; Hadler-Olsen *et al*, 2013; Said *et al*, 2014).

In patients with CRC the roles and functions of MMPs, their diagnostic sensitivity as circulating biomarkers and utility as pharmacological targets is a promising and a challenging area for future investigations (Mysliwiec and Ornstein, 2002; Herszenyi *et al*, 2012). Even though the prognostic value of some MMPs is a matter of literature debate, several studies and meta-analyses have revealed the association among overexpression of MMP-1, MMP-2, MMP-7, MMP-9 and MMP-13 with worse outcome, poorer overall and progression-free survival, suggesting them as prognostic indicators and potential target for treatment in CRC patients (Li *et al*, 2013; Shi *et al*, 2013; Said *et al*, 2014; Sun *et al*, 2015; Hoelzle *et al*, 2016; Salem *et al*, 2016).

Although the advantages of using plasma *versus* serum for MMP biomarker analyses (especially in patients with CRC) are not new (Gerlach *et al*, 2007; Mannello, 2008; Gimeno-Garcia *et al*, 2015), and even if some studies shed light on the poor diagnostic/prognostic accuracy of serum MMP in patients with CRC (Liu *et al*, 2014; Otero-Estevez *et al*, 2015), a plethora of studies measured MMP levels using mostly serum, which can lead to spurious results due to 'pre-analytical flaws' (Mannello, 2008; Jung *et al*, 2009).

Accordingly, in this issue of the *British Journal of Cancer*, Jonsson *et al* (2016) describes the effects of blood sampling on the concentrations of Collagenase-1 (EC 3.4.24.7, MMP-1), Gelatinase-A (EC 3.4.24.24, MMP-2), Matrilysin (EC 3.4.24.23, MMP-7), Collagenase-2 (EC 3.4.24.34, MMP-8), Gelatinase-B (EC 3.4.24.35, MMP-9) and Collagenase-3 (EC 3.4.24.B4, MMP-13) demonstrating significant differences between serum and plasma levels of these proteolytic biomarkers in healthy patients recruited during CRC screening. Jonsson *et al* (2016) found that serum levels of MMP-1, -2, -7, -8 and -9 were significantly higher than those measured in citrate plasma, highlighting that the artificially higher levels of serum MMPs may hamper their diagnostic/prognostic utility in cancer.

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Nevertheless, some investigators continue to neglect the importance of blood sampling, generating relevant pre-analytical pitfalls which undermine the confidence in the conclusions of well-designed cancer research.

Some translational studies (Lim *et al*, 2011; Yu *et al*, 2011) clarified that different blood collecting procedures and the coagulation cascade significantly influence concentrations of both proteins and metabolites in plasma and serum matrices, setting the basis for limiting possible 'pre-analytical flaws' due to blood sampling and handling, especially for cancer biomarker discovery with high-resolution assays (Banks *et al*, 2005).

It was well-appreciated and almost completely assessed that some blood analytes and biomarkers (including also MMPs) are more reliably measured in citrate plasma compared with serum (with or without clot activators; Jung *et al*, 2009), mainly owing to the well-known processes occurring during coagulation (e.g., clot formation removes proteins from the blood matrix; platelet activation releases proteins, cytokines and metabolites; coagulation/fibrinolytic cascade induces a complex network of protease activations; and so on), which finally modify both analyte levels and zymographic profiles in serum samples compared with plasma, inducing possible false conclusions and alteration of the true diagnostic validity of some biomarkers.

The results of Jonsson *et al* (2016) provide further evidence that citrate plasma represents the better choice for evaluating blood MMPs as biomarkers, strengthening previous indications about the misuse of serum (in particular when collected with clot accelerators) able to generate important pre-analytical pitfalls and clinical laboratory biases.

Although the discrepancy between plasma and serum is timely and interesting for biomarker clinical applicability, further robust and novel researches are required to confirm the value of MMPs in patients with CRC.

As promising approaches for CRC screening, faecal mRNA levels of MMP-7 (Takai *et al*, 2009) as well as faecal protein concentrations of MMP-9 (Annahazi *et al*, 2016), may represent new noninvasive tools suitable to distinguish CRC patients from controls and identifying high-risk adenomas, finally providing further evidence of the role of MMPs as tumour markers and early biomarkers of colorectal cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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