Putting a brake on stress signaling: *miR-625-3p* as a biomarker for choice of therapy in colorectal cancer

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Colorectal cancer (CRC) continues to be one of the leading cancer types worldwide [1]. When metastasized the disease is generally incurable and only treated with palliative intent. Metastatic CRC (mCRC) is primarily treated with 5-fluorouracil and leucovorin administered together with either the topoisomerase inhibitor irinotecan (FOLFIRI) or with the alkylating cytostatic oxaliplatin (XELOX/FOLFOX). Although these treatment regimens have significantly improved both disease-free and overall survival, drug resistance is still a major problem, and consequently, only half of the treated mCRC patients obtain an objective response to the selected first-line treatment [2].

Multiple cellular resistance mechanisms in CRC have been described [3]. These include increased drug efflux or drug metabolism as well as alterations in the activity of repair pathways, or, in the case of targeted therapy, alterations in key proteins of the targeted pathway. Despite of this knowledge, the only predictive marker that is widely used in the clinic is the mutational status of the *KRAS* gene; constitutive active mutations in this gene render drugs that target EGFR (an upstream activator of *KRAS*) ineffective [4].

In order to meet today's high expectations of personalized therapy there is thus a dire need to identify predictive markers for the main-stay drugs 5-fluorouracil, SN-38 (the active metabolite of irinotecan) and oxaliplatin.

We recently discovered that high expression of the miRNA *miR-625-3p* in tumors of mCRC patients is associated with more than six-times increased risk of not responding to oxaliplatin-based therapy [5]. miRNAs are a class of 20–22 nucleotides long, noncoding RNAs involved in regulating protein production by suppressing protein synthesis and by initiating mRNA degradation in a sequence-specific manner [6]. miRNAs are involved the control of numerous biological and physiological processes such as cell-cycle control, apoptosis as well as drug response [7].

Not much is known about the cellular functions of *miR-625-3p*. In a recent study by Zheng *et al.*, it was found that *miR-625-3p* increased the migration and invasion of CRC cell lines [8]. These functions were attributed to *miR-625-3p*-mediated downregulation of the SCAI protein encoded by *SCAI* (suppressor of cancer cell invasion).

To gain insight into *miR-625-3p* signaling and its association to oxaliplatin resistance, we have conducted mechanistic studies and molecular profiling in CRC cells with inducible expression of *miR-625-3p* [9]. This showed that *miR-625-3p* causally induces oxaliplatin resistance, and additionally, identified multiple drug response processes dysregulated as a consequence of *miR-625-3p* overexpression. The MAPK family was identified by phosphoproteome profiling as a key pathway class dysregulated by *miR-625-3p*. In addition, and likely

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as a consequence of aberrant MAPK signaling, cells overexpressing *miR-625-3p* appear to bypass down-regulation of cytokine-dependent kinase activity upon oxaliplatin exposure, consistent with the observation that *miR-625-3p* induces increased proliferation and survival in oxaliplatin-containing medium [9].

Explaining *miR-625-3p*-mediated dysregulation of MAPK signaling, MAP2K6 was found to be a direct and functional target of *miR-625-3p*. Consequently, increasing *miR-625-3p* in CRC cells results in decreased MAP2K6 levels [9].

MAP2K6 (also known as MKK6), together with MAP2K3 (MKK3), is responsible for dual phosphorylation and activation of the p38 MAPK family of kinases. p38 α (MAPK14) is the most ubiquitously expressed family member, and its signaling is central for a bewildering range of biological functions including cytokine and growth factor signaling as well as for conveying cellular and environmental stress responses [10].

The role of p38 α in drug response has been intensively studied; although p38 α long has been recognized as a positive regulator of stress-induced apoptosis in transformed and nontransformed cells, studies have also shown its implication in mediating survival and resistance to drug treatment [10].

“This opens up for the possibility of administrating adjuvant anti-miR-625 treatment in combination with oxaliplatin to metastatic colorectal cancer patients with a high miR-625-3p level in order to potentiate the efficacy of therapy.”

MAP2K6 is a substrate for ASK1 (also known as MAP3K5), a MAPK kinase kinase that becomes activated upon genotoxic stress [11]. This, in turn, leads to p38 α phosphorylation and – depending on context – possible induction of apoptosis via downstream effectors. When p38 α function is inhibited, the ability of platinum drugs such as oxaliplatin to induce apoptosis is diminished [9,12,13]. The oxaliplatin resistance observed in *miR-625-3p*-expressing CRC cells is in line with this, as *miR-625-3p* expression leads to decreased levels of MAP2K6, the upstream activator of p38 α and consequently, failure to induce a proper p38 α -dependent apoptotic signaling cascade [9].

During the last two decades, a surprising role for p38 α signaling has emerged that connects growth factor receptor signaling and production of reactive oxygen species (ROS) with the efficacy of combination therapy using EGFR inhibitors together with oxaliplatin.

As a consequence of genotoxic stress, for example, caused by oxaliplatin, p38 α induces apoptosis. This effect is augmented in cells with increasing levels of the H₂O₂ ROS species, possible via enforced

ASK1–MAP2K6 activation [14,15]. Hence, the cellular ROS level appears to be an important determinant for the cytotoxicity of genotoxic drugs [16,17]. Interestingly, external stimuli of growth receptors are associated with the production of low levels of ROS, an activity important for maintenance of normal cellular ROS homeostasis. Abnormal growth receptor pathway activity, such as from constitutive RAS proteins in cancer cells leads to aberrantly increased ROS levels, and can result in a synergistic effect of genotoxic drugs [18]. This interaction between ROS and p38 α -induced apoptosis has been suggested to explain, at least partly, why some patients do not benefit from combination therapy involving oxaliplatin and cetuximab, the latter targeting EGFR, and consequently lowering ROS production [13,18].

Remarkably, in contrast to the p38 α -dependent cytotoxicity of oxaliplatin, SN-38 does not require ROS for induction of apoptosis [13]. On the contrary, p38 α activation is positively associated with resistance to SN-38 *in vitro* and in CRC patients [19]. Although the exact mechanism remains to be elucidated, p38 α -induced survival autophagy is involved in resistance to SN-38 [20], which perhaps explains the opposite effect of p38 α activation on response as compared with oxaliplatin. An intriguing question is whether CRC cells with reduced p38 α signaling as a consequence of *miR-625-3p* upregulation are hypersensitive to SN-38 treatment due to a diminished ability to induce survival autophagy? Further *in vitro* and *in vivo* studies would be needed in order to address this.

Several clinical perspectives of the relationship between *miR-625-3p* and MAP2K6-p38 α stress signalling can be envisioned. Exposure of resistant CRC cells ectopically expressing *miR-625-3p* to an antagonist of *miR-625-3p* (an anti-miR) reactivates oxaliplatin-induced apoptosis [9]. This opens up for the possibility of administrating adjuvant anti-miR-625 treatment in combination with oxaliplatin to mCRC patients with a high *miR-625-3p* level in order to potentiate the efficacy of therapy. An approach that may become reality in the near future as the clinical efficacy and safety of using anti-miRs to reduce the expression of specific disease-associated miRNAs have already been reported [21].

Ultimately, it should also be feasible to develop a simple predictive test for oxaliplatin response in CRC, which could guide the oncologist in selecting an optimal first-line treatment. Such test might be based either on the level or activity of MAP2K6-p38 signaling, or on the expression level of *miR-625-3p* itself. In the latter case, a blood-based test may even be achievable since it is well known that miRNAs

are relatively stable in the circulation compared with other biomolecules such as mRNA [22].

To pave the way for using *miR-625-3p* clinically, it would be necessary to conduct a prospective randomized trial comparing a random assignment to oxaliplatin or SN-38-based therapy against a treatment decision based on the *miR-625-3p* status (or MAP2K6-p38 α) in individual patients. Approximately 20% of mCRC patients have elevated levels of *miR-625-3p* [5], and accordingly, these should be administered an SN-38-based therapy. Such study would determine the clinical benefits, in terms of increased response rates and longer progression-free survival, of a *miR-625-3p*/MAP2K6/p38 α -guided treatment decision.

In conclusion, *miR-625-3p* is a novel promising biomarker that hopefully may benefit CRC patients. By regulating MAP2K6, *miR-625-3p* impacts on the hinge of a central stress signaling pathway, which is part of the cellular response to drug treatment. A functional understanding of the regulation of

drug response pathways does not only enable us to develop biomarkers for today's therapy decisions, but should also facilitate the design of improved drugs for tomorrow's oncological challenges.

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