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Comment on 'TAp63 suppress metastasis via miR-133b in colon cancer cells'

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We have read with great interest the recently published work by Lin *et al* (2014), which provides novel relevant findings about the tumour suppressor role of TAp63 via miR-133b downregulation in colorectal cancer (CRC). Of importance, the authors identified miR-133b as a transcriptional target of TAp63, and showed that the modulation of miR-133b expression is essential for the inhibitory effects of TAp63 in CRC cell migration and invasion. Moreover, they showed that TAp63 is expressed at low levels in CRC and proposed this alteration as a potential cause of miR-133b downregulation, which was previously described by our group in CRC cell lines and patient samples (Bandrés *et al*, 2006). Furthermore, it has been reported that miR-133b has a tumour suppressor role inhibiting cell growth through modulation of the MET signalling pathway (Hu *et al*, 2010), and it has also been described that low expression level of miR-133b correlates with poor clinical outcome in CRC (Akçakaya *et al*, 2011).

Notably, although the findings provided by Lin *et al* (2014) highlight the potential relevance of miR-133b deregulation in CRC progression and metastasis, this issue needs to be fully clarified. A recent publication pointed out that miR-133b contributes to increased CRC cell migration and invasion, and identified CXCR4 as a direct miR-133b target. In that work, Duan *et al* (2014) analysed 31 CRC patients observing miR-133b downregulation in 29 out of 31 tumour samples, and much lower expression in metastatic tumours. The authors proposed that miR-133b could be having a relevant role in CRC invasion and metastasis. However, only 13 out of the 31 CRC patients had metastatic disease (9 with lymph node metastasis and 4 with liver metastasis). Therefore, further studies confirming the role of miR-133b in the metastatic cohort are warranted.

In this line of thinking, we analysed the potential role of miR-133b in CRC progression and metastasis. We quantified the expression pattern of 377 mature microRNAs using Taqman Low Density Arrays (TLDAs) panel A (Applied Biosystems, Grand Island, NY, USA) in primary and paired metastatic tissues from 17 CRC patients, 12 with liver metastasis and 5 with lung metastasis previously reviewed by a pathologist (FR) to further confirm the diagnosis. All samples were taken anonymously and the ethical committee and institutional review board approved the project. Analysis of relative gene expression data was performed using the $2^{-\Delta Ct}$ method and U6B was used as internal control. Downregulation was considered when expression in the metastatic tissue showed at least three-fold decrease compared with its paired primary CRC tissue.

Interestingly, we found miR-133b significantly downregulated in liver metastatic tissues compared with their paired primary CRC tissues ($P<0.001$). We observed that miR-133b was markedly downregulated in all the 12 CRC liver metastatic tissues analysed. Furthermore, we found lower miR-133b levels in lung metastasis compared with their paired primary CRC tissues although significance was not achieved in this case. In fact, we unexpectedly observed that only two out of the five CRC lung metastatic samples showed miR-133 downregulated and even in one of those cases miR-133b expression was found increased. Moreover, miR-133b showed almost five-fold lower expression levels in liver metastatic tissues compared with lung metastatic tissues.

Altogether, we have confirmed the potential relevance of miR-133b in a larger cohort of CRC patients with liver metastasis and the proposed role for miR-133b in metastatic CRC. In addition, our results would indicate that miR-133b downregulation is more specific of liver CRC metastasis, which indicates that miR-133b might be having a potential role determining the metastatic niche, although further studies are warranted to clarify this issue.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Akçakaya P, Ekelund S, Kolosenko I, Caramuta S, Ozata DM, Xie H, Lindforss U, Olivecrona H, Lui WO (2011) MiR-185 and miR-133b deregulation is associated with overall survival and metastasis in colorectal cancer. *Int J Oncol* **39**: 311–318.

Bandrés E, Cubedo E, Agirre X, Malumbres R, Zárate R, Ramirez N, Abajo A, Navarro A, Moreno I, Monzó M, García-Foncillas J (2006) Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. *Mol Cancer* **5**: 29.

Duan FT, Qian F, Fang K, Lin KY, Wang WT, Chen YQ (2014) MiR-133b, a muscle-specific microRNA, is a novel prognostic marker that participates in the progression of human colorectal cancer via regulation of CXCR4 expression. *Mol Cancer* **12**: 164.

Hu G, Chen D, Li X, Yang K, Wang H, Wu W (2010) MiR-133b regulates the MET proto-oncogene and inhibits the growth of colorectal cancer cells in vitro and in vivo. *Cancer Biol Ther* **10**: 190–197.

Lin CW, Li XR, Zhang Y, Hu G, Guo YH, Zhou JY, Du J, Lv L, Gao K, Zhang Y, Deng H (2014) TAp63 suppress metastasis via miR-133b in colon cancer cells. *Br J Cancer* **110**: 2310–2320.

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Response to comment on 'TAp63 suppress metastasis via miR-133b in colon cancer cells'

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The work by Cristobal *et al* (2014) is an interesting study that builds on our and other recent work implicating downregulation of miR-133b in CRC and correlate with CRC metastasis (Lin *et al*, 2014). To further confirm the role of miR-133b in the metastatic cohort, expression pattern of 377 mature microRNAs were detected in primary and paired metastatic tissues from 17 CRC patients. The results showed that miR-133b significantly downregulated in liver metastatic tissues compared with their paired primary CRC tissues, and markedly downregulated in all the 12 CRC liver metastatic tissues. The major concern is why the authors only chose to analyse miR-133b expression between liver metastatic tissues and their paired primary CRC tissues? A recent study identified the microRNA signature between colorectal recurrences to lymph nodes and liver and between colorectal liver metastasis and primary hepatic tumour (Drusco *et al*, 2014). Wang *et al* (2013) also chose to reveal has-miR-337-3p expression in the metastatic tissues, lymph node metastatic tissues, and the primary gastric cancer tissues. Therefore, we think it would be more persuasive if this study also analyses miR-133b expression in lymph node metastatic tissues.

REFERENCES

Cristobal I, Madoz-Gurpide J, Martin-Aparicio E, Carames C, Aguilera O, Rojo F, Garcia-Foncillas J (2014) Comment on 'TAp63 suppress metastasis via miR-133b in colon cancer cells'. *Br J Cancer* **110**: 2310–2320.

Drusco A, Nuovo GJ, Zanesi N, Di Leva G, Pichiorri F, Volinia S, Fernandez C, Antenucci A, Costinean S, Bottoni A, Rosito IA, Liu CG, Burch A, Acunzo M, Pekarsky Y, Alder H, Ciardi A, Croce CM (2014) MicroRNA profiles discriminate among colon cancer metastasis. *PLoS One* **9**(6): e96670.

Lin CW, Li XR, Zhang Y, Hu G, Guo YH, Zhou JY, Du J, Lv L, Gao K, Zhang Y, Deng H. (2014) TAp63 suppress metastasis via miR-133b in colon cancer cells. *Br J Cancer* **110**(9): 2310–2320.

Wang Z, Wang J, Yang Y, Hao B, Wang R, Li Y, Wu Q (2013) Loss of has-miR-337-3p expression is associated with lymph node metastasis of human gastric cancer. *J Exp Clin Cancer Res* **32**(1): 76.

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Comment on 'Pre-operative nomogram for the identification of lymph node metastasis in early cervical cancer'

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Sir,

We read with interest the article by Kim *et al* (2014), highlighting important challenges in pre-operative lymph node staging in early-stage cervical cancer.

From our point of view the article warrants further debate:

(a) First question: Is there really a commitment to offer lymphadenectomy to all women with early-stage cervical cancer?
(b) Second question: Is there an algorithm or nomogram to assess the nodal risk involvement to better select women who can really benefit from retroperitoneal surgical staging?
(c) Third question: What is the impact of those tools in the decision-making process in women desiring to preserve fertility?

Presence of nodal metastasis is one of the most important risk factors for recurrence in surgically treated patients with early-stage cervical cancer (Sevin *et al*, 1995). The incidence of pelvic lymph node metastasis in stage IB cervical cancer ranges from 11.5 to 21.7% (Morice *et al*, 1999; Gien and Covens, 2009).

In the series of Kim *et al* (2014), 81.6% of patients were diagnosed with cervical cancer with a low risk for nodal involvement. In such patients, PET/CT seems to have limited impact as it is not sensitive enough to assess lymph node status.

PET/CT has a spatial resolution limit equal to or lower than 5 mm, and considering its low sensitivity for detection of lymph node metastasis, in our opinion, it seems to have a limited impact on the management of early-stage cervical cancer patients with tumour size smaller than 4 cm.

In a previous series of selected early-stage cervical cancer patients ($n = 159$), we found a rate of nodal metastases of 8% in women with clinical tumour size less than 2 cm, and 34% in women with tumour diameter 2–4 cm. In this low-risk population 56% of lymph node-positive patients (34 of 61) demonstrated nodal metastatic deposits < 5 mm. PET/CT sensitivity and negative predictive values (NPVs) were 32.1 and 69.2%, respectively (Signorelli *et al*, 2011).

In our recent study (Crivellaro *et al*, 2012), we found the mean MTV (Metabolic Tumour Volume) of patients with tumours clinically larger than 2 cm to be significantly higher (17.0 ml) than that of patients with smaller tumours (6.4 ml), and the rate of nodal metastases in the first and second groups was 33 and 9%, respectively.

Sentinel node (SN) biopsy has gained attention, because emerging data from retrospective studies have highlighted its prognostic impact on survival from micrometastases (Gortzak-Uzan *et al*, 2010). A recent study (Cibula *et al*, 2012) confirmed preliminary data in a large cohort of 645 patients with early-stage cervical cancer undergoing surgical treatment, including SN biopsy. The presence of micrometastasis in SN was associated with significantly reduced overall survival, which corresponded to the survival in patients with macrometastasis.

This scenario underlines the importance of micrometastasis in early-stage disease, raising important considerations for the subgroup of young women candidates for conservative treatment, in whom identification of algorithms and pre-operative nomograms incorporating SN should be useful.

In our Department, women with tumours greater than 2 cm (stage 1B1–IIA1) with negative nodal involvement after pelvic lymphadenectomy and SN mapping are offered neoadjuvant chemotherapy with three cycles of TIP (Cisplatin, Ifosfamide and Paclitaxel). In cases of optimal pathologic response (CR + PR1) we perform a simple trachelectomy.

Because micrometastasis seems to be an independent prognostic factor for survival in early-stage disease, and considering that negative pre-operative workup for nodal metastasis still has a high false-negative rate, SN mapping must be incorporated in fertility-sparing surgery.

In case of bulky disease, achievement of an optimal pathologic response after neoadjuvant chemotherapy seems to be a strong independent predictor of survival even in conservative approach (Marchiole *et al*, 2011); therefore, considering the higher risk for relapse, in case of suboptimal response (PR2 o more) we omit a conservative approach in favour of radical surgery.

The debate on whether or not to perform radical lymphadenectomy in early cervical cancer appears strange, considering the outcome in breast cancer, as the publication of the results of Z0011 showed no outcome differences between axillary dissection and no further axillary surgery in patients with positive SN, raising doubts on the role of SN biopsy. The SOUND randomised trial comparing SN biopsy with mere observation in patients with a negative axillary ultrasound who are small breast cancer candidates for breast-conserving surgery is ongoing at the European Institute of Oncology of Milan (Gentilini and Veronesi, 2012).

Prospectively, application of integrated imaging and SN algorithms could be 'traded-off' between no nodal dissection and systematic lymphadenectomy in patients with early-stage cervical cancer, minimising morbidity and the false-negative rate of SN mapping and tailoring the treatment of patients with early-stage cervical cancer.

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REFERENCES

Cibula D, Abu-Rustum NR, Dusek L, Zikán M, Zaal A, Sevcik L, Kenter GG, Querleu D, Jach R, Bats AS, Dyduch G, Graf P, Klat J, Lacheta J, Meijer CJ, Mery E, Verheijen R, Zweemer RP (2012) Prognostic significance of low volume sentinel lymph node disease in early-stage cervical cancer. *Gynecol Oncol* **124**(3): 496–501.

Crivellaro C, Signorelli M, Guerra L, De Ponti E, Buda A, Dolci C, Pirovano C, Todde S, Fruscio R, Messa C (2012) 18F-FDG PET/CT can predict nodal metastases but not recurrence in early stage uterine cervical cancer. *Gynecol Oncol* **127**(1): 131–135.

Gentilini O, Veronesi U (2012) Abandoning sentinel lymph node biopsy in early breast cancer? A new trial in progress at the European Institute of Oncology of Milan (SOUND: Sentinel node *vs* Observation after axillary UltraSouND). *Breast* **21**(5): 678–681.

Gien LT, Covens A (2009) Lymph node assessment in cervical cancer: prognostic and therapeutic implications. *J Surg Oncol* **99**: 242–247.

Gortzak-Uzan L, Jimenez W, Nofech-Mozes S, Ismiil N, Khalifa MA, Dubé V, Rosen B, Murphy J, Jaframboise S, Covens A (2010) Sentinel lymph node biopsy *vs* pelvic lymphadenectomy in early stage cervical cancer: is it time to change the gold standard? *Gynecol Oncol* **116**(1): 28–32.

Kim DY, Shim SH, Kim SO, Lee SW, Park JY, Suh DS, Kim JH, Kim YM, Kim YT, Nam JH (2014) Preoperative nomogram for the identification of lymph node metastasis in early cervical cancer. *Br J Cancer* **110**(1): 34–41.

Marchiole P, Tigaud JD, Costantini S, Mammoliti S, Buenerd A, Moran E, Mathevet P (2011) Neoadjuvant chemotherapy and vaginal