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Comment on: α-smooth muscle actin expression and desmoplastic stromal reaction in pancreatic cancer: results from the CONKO-001 study

I H Sahin*[,1] and B Uzunparmak[2]

[1]Department of Medicine, Mount Sinai Icahn School of Medicine, St Luke's Roosevelt Hospital Center, New York, NY, USA and [2]Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Sir,

We read with great interest the recent study by Sinn *et al* (2014) that demonstrated significantly increased α-smooth muscle actin (α-SMA) expression in pancreatic cancer stroma, which was correlated with worse survival outcomes in patients who underwent tumour resection and did not receive any adjuvant treatment. The authors also showed that dense stroma in the tumour microenvironment is associated with better outcomes in pancreatic cancer patients. We would like to discuss further points about the relationship between increased α-SMA expression and worse outcomes in pancreatic cancer patients.

Increased Sonic Hedgehog signalling in both tumour cell and tumour stroma has been found to be related to increased α-SMA expression (Bailey *et al*, 2008). Sonic Hedgehog signalling has also been demonstrated to be involved in pancreatic cancer stem cell development (Takebe *et al*, 2010), and the Sonic Hedgehog transcript was shown to be increased four-fold in the general pancreatic cancer cell population, but 46-fold in CD44 + CD24 + ESA + pancreatic cancer stem cells (Lee *et al*, 2008). Moreover, pancreatic cancer stem cells have a 100-fold greater tumour-initiating capability compared with non-stem pancreatic cancer cells (Li *et al*, 2007). Therefore, increased α-SMA expression actually may indirectly indicate an increased pancreatic cancer stem cell population that is directly related to tumour growth and metastatic activity (Hermann *et al*, 2007), and the associated increased Sonic Hedgehog signalling in the tumour microenvironment.

A recent study showed that stromal elements that respond to tumour growth restrain the tumour growth, and that inhibition of the stromal response induces more aggressive tumour behaviour and disease progression via increased angiogenesis (Rhim *et al*, 2014). Similarly, increased cell proliferation in pancreatic intraepithelial neoplasia has been observed upon inhibition of hedgehog signalling in the tumour stroma (Lee *et al*, 2014). Moreover, a phase II clinical trial investigating the role of the Sonic Hedgehog signal inhibitor, saridegib combined with gemcitabine, was terminated early due to worse survival outcomes in the treatment arm compared with the placebo plus gemcitabine arm (Lou, 2014). A more recent clinical trial also failed to demonstrate any benefit of inhibiting the Sonic Hedgehog pathway; even more strikingly, there was no significant effect on pancreatic cancer stem cells either (Kim *et al*, 2014).

Altogether, elevated α-SMA expression in tumours may be indirectly related to survival outcomes and rather it may be a sign of increased cancer stem cell population, as studies have shown no benefit upon inhibition of desmoplasia. Further studies are required to enlighten the exact relationship between cancer stem cells and the tumour microenvironment.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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*Correspondence: Dr IH Sahin; E-mail: md.ibrahim.halil.sahin@gmail.com
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Sleep duration and breast cancer risk in the breast cancer detection demonstration project follow-up cohort: true associations or bias?

W-S Yang*[,1,2], X Wang[1,2], Q Deng[1,2], H Zhao[1,2] and W-Y Fan[1,2]

[1]Department of Social Science and Public Health, School of Basic Medical Science, Jiujiang University, No. 17, Lufeng Road, Jiujiang 332000, China and [2]Jiangxi Province Key Laboratory of Systems Biomedicine, Jiujiang University, No. 17, Lufeng Road, Jiujiang 332000, China

Sir,

We read with interest the recent publication by Qian *et al* (2014). The authors examined the risk of incident breast cancer (BC) associated with sleep duration using data from Breast Cancer Detection Demonstration Project follow-up cohort, and found a null association between sleep hours and overall BC. They also reported risk estimates for BC according to different molecular subtypes of BC, and suggested a decreased risk for estrogen receptor (ER) + progesterone receptor (PR) + BC with shorter sleep duration. The information provided is of interest as the relationship between sleep and BC is of increasing concern. However, we would like to raise several concerns related to this paper.

First, the validity of the sleep questionnaire used in the study is unclear. As self-reported sleep duration is potentially subject to misclassification, and the exposure variable (sleep hours) was categorical, even random misclassification may have led to bias in any direction (Rothman *et al*, 2013). A previous validation study (Girschik *et al*, 2012) concluded that a three-item sleep questionnaire that is similar to one used in the study by Qian *et al* (2014) and typically employed in other epidemiologic sleep studies exhibited a poor agreement with objective measures of sleep as assessed using actigraphy (kappa coefficients ranging from −0.19 to 0.14). Thus, the misclassification bias for exposure data in their study cannot be ruled out. Moreover, the data on sleeping habits in

the analysis was about the information of most recent year at baseline, which may not reflect the long-term sleeping habits.

Second, the lack of adjustments for other sleep factors in the analysis could have confounded their results. A plausible biological model, that is, light exposure at night (LAN)–melatonin–BC (Stevens and Davis, 1996) may interpret how poor sleep can directly affect the development of BC. In this hypothesis (Stevens and Davis, 1996), LAN is deemed to be associated with an increased risk for incident BC by decreasing the melatonin release by pineal gland. However, melatonin release rely on the light/dark cycle (Blask, 2009) rather than on sleep duration only, and other sleep characteristics such as sleep quality, LAN, the use of sleeping pills, habitual timing of sleep, and night waking times may also influence the outcome for incident BC (Yang *et al*, 2014); therefore, the potential confounding bias may exist. In addition, as the exposure data collection is described in a concise manner, it is unclear whether the 'sleep hours' is a real sleep duration at night-time or hours spent in bed.

Third, the findings, particularly for ER + PR + BC, seemed to somewhat contradict the current possible biological mechanism, that is, LAN–melatonin–BC (Stevens and Davis, 1996). Such a discrepancy, as acknowledged by authors, could be due to chance. However, other factors, as mentioned above, including poor quality of exposure data (see comment 1) and lack of consideration for other sleep factors (see comment 2) may partly explain this controversy, because if the LAN–melatonin–BC hypothesis is true, as mentioned above, sleep is not necessarily required for synchronisation of the endogenous circadian rhythm (Blask, 2009), although melatonin release depends on a stable 24-h light/dark cycle, other sleep patterns such as habitual timing of sleep, waking up frequency, night-time lighting conditions, and sleep quality may also affect melatonin release (Yang *et al*, 2014).

Altogether, although this cohort study provided new information on the relationship between sleep duration and BC risk, the quality of exposure assessment and other covariates relating to sleep should be considered when interpreting results. With this in mind, we now are establishing a large population-based case–control study to assess the risk of BC associated with sleeping factors and other potential risk factors in Jiujiang City, China. As for exposure assessments, we are systematically collecting sleeping factors including sleep quality, LAN, night/shift work, the use of sleeping pills, sleep hours, habitual timing of sleep, and frequency for night-time wakings using the self-made 22-item sleeping factors questionnaire (SFQ). We have conducted a pilot study to check the validity and reproducibility of SFQ used in our project. In the pilot study, the SFQ was interview-administered twice, ~1 year apart, and participants were also asked to complete a 'sleeping diary' for 30 consecutive days every quarter over this same year accounting for seasonal effects. We examined the validation by comparing the average measures between two SFQs and four sleeping diaries, and examined the 1-year stability of SFQ by comparing the measures in the two SFQs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DISCLAIMER

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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*Correspondence: Dr W-S Yang; E-mail: wanshuiyang@gmail.com
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Reply to 'Sleep duration and breast cancer risk in the breast cancer detection demonstration project follow-up cohort: true associations or bias?'

X Qian*[,1], L A Brinton[2], C Schairer[3] and C E Matthews[1]

[1]*Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, Maryland, USA;* [2]*Hormonal and Reproductive Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, Maryland, USA and* [3]*Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, Maryland, USA*

Sir,

We thank Yang *et al* (2015) for their interest in and thoughtful review of our study. We agree with the correspondents that certain limitations should be considered when interpreting findings on sleep duration and breast cancer risk. As we acknowledged in our paper, self-reported sleep duration involving measurement error could lead to misclassification of our main exposure. We agree with Yang *et al* that misclassification can lead to bias in either direction. However, in our case, we believe the bias is more likely to be towards the null. A validation study by Lauderdale *et al* (2008) compared self-reported sleep duration to an objective measure (actigraphy), and found that the validity of self-reported sleep varied by the amount of sleep recorded. In general people tended to over-report their sleep duration, but the extent of over-reporting increased as sleep duration decreased. Therefore, short sleepers were at a higher risk of being misclassified as normal or long sleepers, which might have led to an inability to detect an increased risk of breast cancer among such individuals. Of course, regardless of the direction of the bias, misclassification of exposure is an important problem to consider, and we appreciate that Yang *et al* highlighted this particular element of our report. Moreover, we also agree with the correspondents that it is important to measure sleep at different time points in order to get a better estimate of long-term sleep duration, and to consider other sleep characteristics and sleep-related factors, such as sleep quality and exposure to light at night.

We are aware of the discrepancy between our findings and the melatonin hypothesis, which suggests that short sleep duration is associated with decreased levels of melatonin. Because melatonin is a molecule with anti-oestrogenic effects, decreased levels of melatonin may increase the risk of ER + tumours (Blask, 2009). We found no association between sleep duration and hormone receptor-positive tumours, which is consistent with the only two studies that examined sleep duration in relation to breast cancer subtypes defined by hormone receptor status. Notably, both studies, an Australian case–control study and a study in the Women's Health Initiative, showed no relationship between sleep duration and ER + tumours (Girschik *et al*, 2013; Vogtmann *et al*, 2013). In contrast, we found an increased risk associated with short sleep durations for hormone receptor-negative breast cancers. Although we cannot exclude the possibility that our finding is due to bias or chance, we believe that there are biological mechanisms that support this observed association. For example, short sleep and sleep deprivation have been associated with factors that may influence breast cancer risk