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European Journal of Cancer 37 (2001) 668–670

European Journal of Cancer

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Letters

# Comments on: *Involvement of adenomatous polyposis coli (APC) β-catenin signalling in human breast cancer*, Jönsson M, Borg Å, Nilbert M, Andersson, T. *Eur J Cancer* 2000, **36**, 242–248

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Received 24 October 2000; accepted 27 October 2000

Jönsson and colleagues were able to provide indirect evidence of the involvement of β-catenin signalling in human breast cancer, by showing that 13% of primary breast tumours had increased cytosolic β-catenin. However, they found no correlation between the increased β-catenin levels with increased c-MYC levels, the latter being a downstream transcriptional target of the β-catenin signalling pathway. They also were unable to demonstrate the presence of β-catenin in the nuclei of breast tumour cells by immunohistochemistry, due to the poor performance of the antibody used [1].

It would indeed be convincing to demonstrate the physical presence of β-catenin in the nuclei of breast tumours. We have used the anti β-catenin antibody CAT-5H10 from Zymed, and were able to demonstrate strong β-catenin staining in the nuclei of both colon and ovarian carcinomas. However, in 71 cases of human breast tumours (33 invasive duct not otherwise specified, 16 invasive lobular, 21 mixed ductal-lobular, 1 ductal carcinoma *in situ* (DCIS) only), not one tumour showed any nuclear β-catenin staining (Fig. 1).

The focus of our laboratory is on the transmembrane glycoprotein mucin-1 MUC1, which is found overexpressed, both on the plasma membrane, and in the cytosol, in virtually all human breast cancers. The study by Yamamoto and colleagues [2] which demonstrated that MUC1 and β-catenin could interact, led us to investigate whether the aberrant presence of MUC1 in the cytosol could be interfering with the interaction β-catenin with adenomatous polyposis coli gene product (APC), resulting in decreased β-catenin degradation, and subsequent transport β-catenin into the nucleus. Our inability to demonstrate β-catenin in the nuclei of breast tumours, coupled with the finding of Li and associates [3] that overexpression of MUC1 "has little if any effect on the distribution of β-catenin in the cytoplasm and nucleus", has led us to redirect our studies.

Jonsson's finding that c-MYC and β-catenin levels do not correlate, and our inability to demonstrate nuclear β-catenin in breast cancer with an antibody known to be able to detect nuclear β-catenin in colon cancer, suggests that β-catenin signalling is not a major factor in

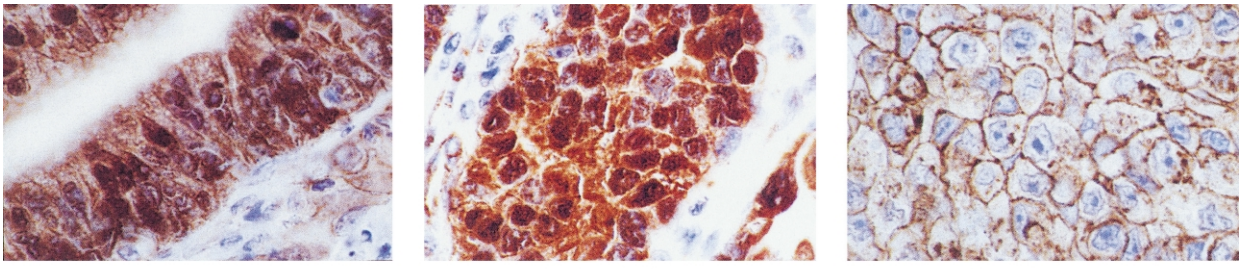


Fig. 1. We were unable to detect β-catenin in the nuclei of any of the breast cancer cases examined. This was in stark contrast to the cases of colon and ovarian cancer.

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PII: S0959-8049(00)00425-1

breast cancer tumorigenesis. Since Jönsson and colleagues have a protocol for isolation of the nuclei of primary breast tumours, perhaps it would be profitable to prepare a nuclear lysate, which could be analysed by Western blotting for the presence of the β-catenin protein. Hopefully, this could definitively answer the question once and for all.

**Acknowledgements**

This work was supported by the Alberta Cancer Board.

**References**

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# Response

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Received 6 December 2000; accepted 12 December 2000

In a letter to the Editor published in this issue of the *European Journal of Cancer* pp. 669–670 Rahn and Hugh illuminated the difficulties in detecting β-protein in the nuclei of breast tumour cells. These authors were unable to demonstrate the nuclear localisation of β-catenin in 71 investigated breast tumours (33 invasive ductal, 16 invasive lobular, 21 mixed ductal-lobular, ductal carcinoma *in situ* (DCIS), but they were successful in detecting immunoreactive β-catenin in the nucleus of colon and ovarian carcinomas.

We have previously reported the same difficulties in nuclear staining of β-catenin protein in breast tumour cells, and Rahn and Hugh have recommended that we use an alternative method to circumvent this problem. The method they suggested is based on isolation of the nuclei from primary breast tumour cells and subsequent western blot analysis of the nuclear lysate for presence of β-catenin. This might work well for larger tumours, but it has an obvious shortcoming in that the majority of breast tumours removed from patients are small, therefore isolated nuclei will not provide sufficient protein to allow detection by western blotting. In addition, there is a risk that the nucleic β-catenin protein will be contaminated with cytosolic β-catenin during cell lysis and isolation of nuclei from the cell extract, which would make it impossible to interpret the results.

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It is interesting that Rahn and Hugh failed in their attempts to stain nucleic β-catenin protein in breast tumour cells, whereas they were able to detect this protein in the nuclei of colon tumours when using the same antibody. This suggests that the absence of β-catenin in nuclei of breast tumour cells might not be due to technical problems, but instead reflect the diversity of β-catenin signalling pathway between breast and colon cancer. In support of this idea, several groups have provided evidences that the Wnt/β-catenin signalling pathway in breast cancer does not follow the pattern observed in colon cancer and melanomas [1–3]. In this context, adenomatous polyposis coli (APC) has been recognised as one of the most affected components in the Wnt/β-catenin signalling pathway in colon cancer (80–95%), but not in breast carcinomas (1–5%). Inasmuch as breast and colon epithelial cells are different cell types, and breast cells are influenced by the presence of steriod hormones, the variations in the Wnt/β-catenin signalling pathway indicate that the initiation and promotion of the Wnt/β-catenin signalling in breast cells could be cell-type specific.

The absence of β-catenin in the nucleus of breast cancer cells could also mean that removal of this protein from the nucleus is carried out more effectively by an intact APC protein in breast tumour cells than by a mutated APC in colon tumour cells. In support of this, Henderson has demonstrated that APC protein regulates the subnuclear localisation of β-catenin, that is, he discovered that APC is a nuclear shuttling protein that enters the nucleus and competes with T-cell factor (TCF)/lymphocyte enhancing factor (LEF) for β-cat-