Reviewer's report

Title: Integrating chromosomal aberrations and gene expression profiles to dissect rectal cancer

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Reviewer: Silke Lassmann

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In continuation for their previous studies (Lips EH et al. J Pathol. 2007;212(3):269-77 and Lips EH et al Clin Cancer Res 2008;14(3):772-81), the authors examined mRNA expression profiles in 79 rectal adenomas and carcinomas and compared those to previously determined genome-wide chromosomal alterations within 66/79 cases. With this, the authors set out to identify specific candidate genes differentially regulated in different “tumor stages”, respective adenomas, adenomas with carcinoma “focus”, mixed adenoma and carcinoma, carcinoma without (N0) and with (N+) associated lymph node metastasis.

Comparison of gene expression profiles between adenomas and carcinomas or N0 and N+ carcinomas yielded differential expression patterns, whereas “pure” adenomas and adenomas with carcinoma focus exhibited similar expression profiles.

The authors then proceeded to compare the gene expression profiles with genomic data of SNP-array analyses and revealed that within the “candidate progression genes” up- and down-regulation occurred in parallel to gains/losses of chromosomes 20 and 18. Using supervised clustering analyses, a sub-set of genes within each progression-relevant chromosomal region was identified and of these each a single candidate gene validated by Q-RT-PCR and IHC.

Correlation of Q-RT-PCR and gene expression profiles was generally high, except for the BOP1 gene on chromosome 8q.

The study had two aims, namely 1) to correlate chromosomal aberrations and gene expression profiles and 2) to identify genes involved in rectal carcinogenesis. Due to the wealth of data generated by the various methods and the heterogeneity of tissue samples analysed, some issues need further clarification in order to strengthen the authors’ conclusions:

- Major Compulsory Revisions

1) Correlation of SNP-array and mRNA microarray data:

The conclusions of the study need be strengthened by validation of a larger set of genes located within a specific chromosomal region showing differences between e.g. “adenomas” and “carcinomas”. Thus, it would be worthwhile looking at both well-known (e.g. myc/8q) and more recently described (e.g. Bob1/8q)
genes within the individual chromosomes. Similarly, it is important to check a larger series of “differentially” expressed genes of the mRNA microarrays in the corresponding case-specific SNP-array data sets.

In view of this, it is intriguing that some “stroma”-associated genes turned out to be “up-regulated” (e.g. collagens) in the mRNA microarray analyses. This may simply be due to a difference in the purity of “tissue/cell samples” analysed, even though macrodissection had been performed. “Up-regulation” of genes in mRNA expression profiles may hence not necessarily be caused by malignant progression of the dysplastic epithelial into an invasive tumour cells, but rather by stromal components, such as e.g. lymphocytes, probably not eradicated by macrodissection. This should be discussed.

In contrary, it would be interesting to see whether such potential “stromal genes” are also deregulated at the DNA level.

2) Validation of candidate genes and impact on malignant progression

Both immunohistochemical stainings and corresponding mRNA levels should be shown in representative cases within normal, dysplastic epithelia and invasive carcinomas for selected candidate genes to support their involvement in “rectal carcinogenesis”.

In addition, the authors should discuss the finding that “EFNA1 and VEGF showed increased expression in the more advanced tumour stages” and data in Fig. 4b where VEGF expression appears to be down-regulation of VEGF in node-positive rectal cancers as compared to node-negative rectal cancers.

- Minor Essential Revisions

1) Please check whether for Bob 1 IHC staining it was really “fresh frozen” tumour sections used for “antigen retrieval…boiling…10 min in EDTA buffer”.

2) Please check and expand all figure legends – e.g. legend to fig 1: comparison of which tissue samples is shown (which cases for A and B? also sample 203?); legend to fig. 5: please indicate the IHC scores given and which types of tissue samples are shown. Please change “retention” to “positive expression”.

3) Please state why three housekeeping genes were used for Q-RT-PCR analysis and how relative expression levels were obtained – The 2”(-ddCt) method implies a single reference gene – so how was selection done and was the same housekeeping genes used for all samples?

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

declare that I have no competing interests