Reviewer's report

Title: A biomarker based detection and characterization of carcinomas exploiting two fundamental biophysical mechanisms in mammalian cells

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Reviewer: Jau-Song Yu

Reviewer's report:

In this study, the authors showed the overexpression of DNaseX and TKTL1 in oral squamous cell carcinomas (OSCC) by immunohistochemistry (IHC) and correlated this overexpression with patients’ clinical outcomes, such as recurrence of the tumor and survival rate. Moreover, they used specific mAbs against DNaseX- and/or TKTL1-specific peptide/epitope in conjunction with flow cytometric analysis to detect CD14/CD16 positive monocytes harboring these peptides/epitopes in blood samples from patients with OSCC, breast cancer or prostate cancer, and blood donors/controls, a recently developed method called epitope detection in monocytes (EDIM). They showed that EDIM-Apo10 (a mAb for DNaseX) and EDIM-TKTL1 blood tests allowed a sensitive and specific detection of patients with OSCC, breast cancer and prostate cancer before surgery and in after care. A combined score of Apo10+/TKTL1+ derived from this study led to a sensitivity of 95.8% and a specificity of 97.3% for the detection of carcinomas independent of the tumor entity.

Overall, the results of this manuscript are interesting and may provide a new blood-based method to detect OSCC and other carcinomas in a high sensitivity and specificity. Although the parts of method, introduction and discussion in this paper were well written, the presentation of the experimental data, especially the EDIM-Apo10 and EDIM-TKTL1 blood tests, was not appropriate and needs to be largely improved before the consideration of publication in your journal. For instance, several important experiment results were just described but not really presented/provided by the authors. Followings are comments/critiques raised against this paper by this reviewer.

Major points:

1. (page 13) The authors mentioned “Apo10 (Figure 1a) nuclear overexpression was strongly associated with cancer cells and was not detected in stromal or human normal oral squamous epithelial cells”. Please show the representative IHC image of stromal or human normal oral squamous epithelial cells stained by Apo10.

2. (page 17) The authors mentioned “In order to determine the presence of Apo10 protein epitope in human carcinomas, we performed IHC on 580 human carcinomas derived from 4 different epithelial tumour entities - carcinoma of the lung, colon, bladder, and cervix. Similar to the results observed in OSCC, in the
majority of carcinomas the Apo10 epitope has been detected”. Please summarize and show the data; without showing the real data, it is not appropriate to make this claim.

3. (page 17) The authors mentioned “To determine the expression of Apo10 and TKTL1 in tumour cells, OSCC cell lines BICR3 and BICR56 have been analysed. Single staining of the OSCC cell lines BICR3 and BICR56 in cytopsins served as an additional positive control and confirmed the presence of the Apo10 epitope and expression of TKTL1 in cancer cells (data not shown)”. Please summarize and show the data in another Additional File.

4. (page 17) The authors mentioned “In order to determine the presence of Apo10 protein epitope in human benign cells, we performed IHC on 31 samples of myocarditis patients. A nuclear staining was observed and correlated with apoptosis rate measured by caspase 3 cleavage (data not shown). Furthermore, one cell type with no signs of apoptosis close to the benign colon epithelium showed a very strong nuclear staining (data not shown)”. Please summarize and show the data in another Additional File.

5. (page 18) The authors described the ROC and AUC data from EDIM-Apo10 and EDIM-TKTL1 blood tests and concluded that they are highly sensitive and specific assays for detecting OSCC and recurrence of the tumour. However, simply providing the ROC and AUC data here is not enough to properly evaluate the potential clinical utility of the tow markers. The scores of EDIM-Apo10 and EDIM-TKTL1 for each patient and healthy control should be expressed by, for example, dot plot, and their mean +/- SD should be calculated for the comparison/analysis between patients and controls, just like the comparison/analysis of blood biomarkers using traditional ELISA. These analyses should enable readers to know the “window of the distribution of EDIM-Apo10 and EDIM-TKTL1 scores” in patient and control groups. In addition, the authors should provide at least, for example, the raw data of flow cytometric analysis of two OSCC patients and two healthy controls to clearly illustrate the power of this technology platform to differentiate cancer patients from healthy controls. Such raw data should also include the analysis of breast and prostate cancer patients, and the data can be shown in another new Additional File.

6. (page 18-19) In addition, it is better to create a new table to clearly summarize the results described in the following sentences (in page 18-19) “45 out of 50 patients with OSCC patients showed positive EDIM-Apo10 scores (EDIM-Apo10 positive n = 45/50, 90%; EDIM-Apo10 negative n = 5/50, 10%) and 46 patients showed positive EDIM-TKTL1 scores (EDIM-TKTL1 positive n = 46/50, 92%; EDIM-TKTL1 negative n = 4/50, 8%). Only two patients (n = 2/50, 4%) were negative for both values. Using the combined score (EDIM-Apo10 / EDIM-TKTL1 >227) 47 OSCC patients (n = 47/50, 94%) were positively detected. 4 out of 74 healthy individuals were positive for EDIM-Apo10 (n = 4/74, 5%), 3 out of 74 individuals were positive for EDIM-TKTL1 (n = 3/74, 4%), and 3 individuals were positive for both values (n = 3/74, 4%). 71 of 74 healthy individuals (n = 71/74, 96%) were negative using the combined score”.
7. (page 19) The authors mentioned that “Measurement of EDIM-Apo10 and EDIM-TKTL1 revealed normal scoring levels after R0 resection and convalescence (n = 3)”, suggesting that EDIM-Apo10 and EDIM-TKTL1 could be potential indicators for monitoring the efficacy of cancer treatment. This data is interesting and may be of importance. However, the case number (n=3) is too small to draw meaningful conclusion. It is strongly suggest that the authors should perform more detailed study by including more patients to make solid conclusion; otherwise, this information should not be included in this manuscript. Similar argument also exists for the analysis of samples from the breast cancer (n=3) and prostate cancer (n=6) patients in this manuscript.

8. (page 19/20) The authors have also performed similar studies using samples from breast and prostate cancer patients, which were described in page 19/20 under the paragraph entitled “EDIM-Apo10 and EDIM-TKTL1 blood tests are highly sensitive and specific for detecting patients with breast/prostate cancer”. To show these data in a more convincing way, the authors should provide the scores of EDIMApo10 and EDIM-TKTL1 for each patient by, for example, a dot plot, and their mean +/- SD should be calculated for the comparison/analysis between patients and controls, just like the comparison/analysis of biomarkers using traditional ELISA.

9. (page 22) In Discuss section, the authors mentioned that “The Apo10 epitope is present in neoplastic cells including carcinomas, sarkomas, glioblastomas, lymphomas, and leukemias, whereas on few benign cells with and without induction of apoptosis show this epitope.”. Please indicate appropriate references for this description. Similar comments also exist for the description about the overexpression of TKTL1 in different cancer types.

Minor points:
1. The antigen sequence for mAb Apo10 and anti-TKTL1 antibody used in this study should be provided if they are known or applicable.

2. (page 8/9) In the “Staining procedure and quantification of IHC” section, the algorithms for scoring IHC data of TKTL1 and Apo10 are different. Please explain why different algorithms were applied for the two Abs when they were used to stain the same sample set.

3. It seems that the detailed protocol(s) for preparation of CD14/CD16 positive monocytes from blood samples for EDIM-Apo10 and EDIM-TKTL1 assays have been described in the authors’ previous publications. If yes, please clearly indicate this fact in the appropriate position of the method section. If not, the authors should describe them in detail in this manuscript.

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

Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests