World Cancer Research Journal WCRJ 2015; 2 (1): e467

COMMENTARY:
CRUCIAL, BUT STILL POORLY KNOWN, KEY POINTS REGARDING DESIGN AND CONSTRUCTION OF TMA

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The technology of tissue microarrays (TMA) allows the simultaneous analysis of hundreds of tissue samples on a single slide using standard light microscopy, immunohistochemistry or in situ hybridization. Basically, this technology facilitates and speeds up the characterization of the expression of new proteins[1]. With the help of statistical analysis correlating patterns of expression with the clinical information, the TMA may define the prognostic significance of tumor markers.

Well recognized advantages of TMA include the following items: 1) simultaneous testing of the expression of new markers on a large number of samples, allowing the evaluation and comparison of the expression profile of markers on a large number of neoplastic, pre-neoplastic or normal tissues; 2) testing an entire and large cohort of cases with a given antibody on a single glass; 3) identical conditions of preparation for all tissues analyzed; 4) excellent correlations between TMA and whole sections; 5) costs reduction for the evaluation of immunohistochemistry or in situ hybridization in large numbers of tumor samples or normal tissues compared to standard techniques with whole sections; 6) easy shareability for performing multi-institutional studies[2].

In their paper entitled "Use of tissue microarrays in translational research" Sabatino et al[3] describe the use of TMA in clinical and research laboratories with special emphasis on tumor microenvironment investigation. The Authors also discuss about the benefits and pitfalls of TMA technology. In conclusion, they recommend that TMA technology should be used with markers of a high expression and a more homogeneous staining pattern, while whole section slides is a superior method for markers which are expressed in few cells and which show a focal and heterogeneous staining pattern.

Actually, the TMA technique has some significant weaknesses (Figure 1). Studies based on TMA, in particular, depend on the good quality of TMA construction and are influenced by the use of appropriately validated antibodies and standardized laboratory techniques. The "workflow" used to validate the TMA should ideally include, as a first phase, studies aimed at defining the standard operating procedures (SOP) for each of the steps involved. In the second phase, ad hoc studies should be carried out to verify the reliability of the SOP developed in terms of precision, accuracy and reproducibility, considering as reference values the measurements obtained with the staining of whole sections. A crucial step for the success of the construction of a TMA is to determine the appropriate size of the case study to analyze. The next step is the collection of cases. This requires careful planning to maximize the number of projects that will use the TMA, the appropriateness of the controls, the diagnosis confirmation and classification, and the staging of cases. If a project involves patients from a multi-year period, the

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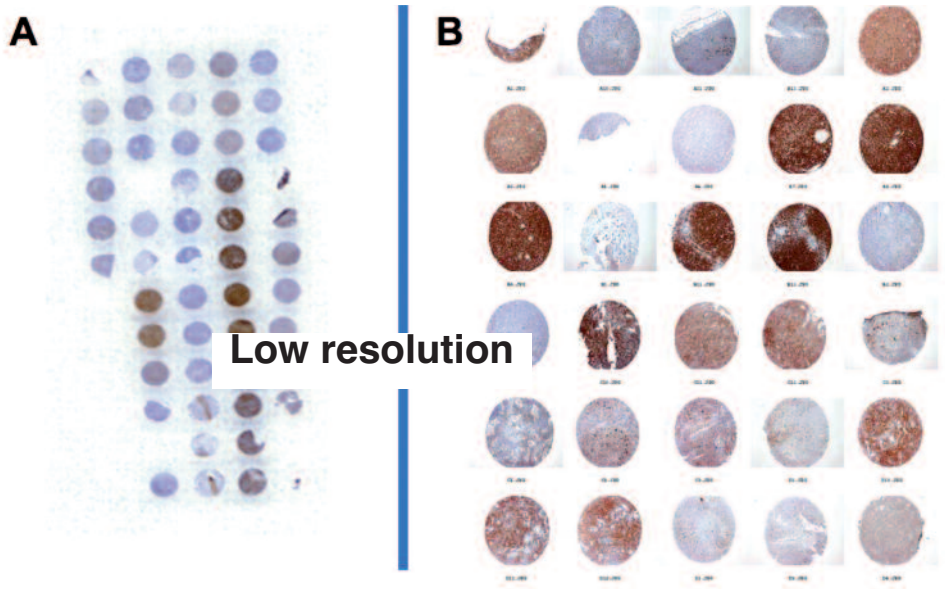

Figure 1. (**A**) In this TMA, some small disks of tissue are damaged or lost. This common event may be overcome by harvesting duplicate or triplicate recipient blocks. (**B**) This is a composite microphotograph in which images are placed with definite arrays coordinates

researcher must ensure that the characteristics of the selected patients in the early stages of the study are comparable to those of the patients involved in the final stages of the study. Since the TMA technology facilitates the study of large cohorts, researchers often use archival tissues preserved in a wide span of time, often with changes in processing techniques over time. Even if a tissue fixed in formalin and embedded in paraffin can maintain its antigenicity for many decades, the researchers must control that there is no correlation between staining intensity and age of the archived tissue. The layout is built using an Excel file and assigning a case to each cell of the spreadsheet. The positions of the control tissues should be randomized and interspersed with the other cores. If all control tissues are in an area of the TMA, the interpretation may be influenced unless an image analyzer is used. It may be useful to organize the core blocks in order to reduce the possibility of error during construction and allow visual monitoring during the analysis.

According to Sabatino and Colleagues, TMA does not represent the routine method for most pathologists. Conventional whole sections could be preferred for clinically validating potential biomarkers. However, we believe that TMA technology, once validated, represents an excellent alternative to other tools, such as cell lines, to implement external quality assessment studies focused on the whole process of biomarker determination[4].

Acknowledgements:
Supported in part by an Institutional grant from Centro di Riferimento Oncologico Aviano for a project on “Molecular Pathology”.

Conflict of Interests:
The Authors declare that they have no conflict of interests.

REFERENCES

1. Kononen J, Bubendorf L, Kallioniemi A, Bärlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP. Tissue microarrays for high-throughput molecular profiling of tumour specimens. Nat Med 1998; 4: 844-847.
2. The College of American Pathologists. www.cap.org
3. Sabatino R, Santonastaso C, Franco R, Scognamiglio G. Use of tissue microarrays in translational research. WCRJ 2014; 1: e356
4. Verderio P, Carbone A. Tissue microarrays for immunohistochemical determination of oncological biomarkers. Virchows Arch 2009; 454: 353-354.