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Author's view

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Abstract

We determined the proportions of epithelioid-like and sarcomatoid-like cellular entities within malignant pleural mesothelioma samples, by deconvolution of their transcriptomes. These proportions are associated with prognosis and may guide therapeutic strategies. This novel approach describes both intra- and inter-tumor heterogeneity and provides a new way to think about cancer pathology.

Keywords

Mesothelioma, Tumor heterogeneity, Deconvolution method, Molecular component, Molecular classification.

Number of words: 873

Main manuscript

Malignant Pleural Mesothelioma (MPM) is a rare thoracic tumor, emblematic of the occupational cancers due to its strong link to asbestos exposure. MPM is also one of the most dreadful cancer and current treatment options are not curative, with very few exceptions [1]. As for most cancer types, clinical trials have highlighted MPM diversity in terms of prognosis and patients' response to anti-cancer agents, suggesting an underlying tumor heterogeneity [2]. Better understanding inter and intra-tumor heterogeneity is thus of great importance for the identification of therapeutic strategies, and for the implementation of precision medicine, with the aim and hope to cure patients.

In a previous study, we have defined a robust molecular classification defining two molecular subtypes, related to prognosis and differing by their engagement in the epithelial-mesenchymal transition (EMT) [3]. More recently, classifications in four subtypes also related to prognosis and partly to genetic alterations were also proposed [4, 5]. These subtype classifications take into account the heterogeneity between patient tumors, but not the heterogeneity inside the tumor of each patient. MPM intra-tumor heterogeneity is poorly described at the molecular level, but is well known at the histologic level, with three main histologic types: epithelioid, sarcomatoid and biphasic, the latter being a mix of variable proportion of epithelioid and sarcomatoid MPM. The histologic heterogeneity is even more complex with the characterization of several histologic subtypes [6]. Furthermore, a meta-analysis comparing all of the molecular clusters from six different transcriptomic classifications highlighted only two main groups of highly correlated clusters present in all datasets that corresponded to the most extreme epithelioid and sarcomatoid phenotypes [7]. Intermediate subtypes could simply reflect various cut-offs of a continuum combining epithelioid and sarcomatoid entities, which could be better defined using molecular gradients.

To overcome this limitation, we used WISP (Weighted In Silico Pathology; https://cit-bioinfo.github.io/WISP/), a novel deconvolution method that we developed, that decomposes each bulk MPM molecular profile as a combination of epithelioid and sarcomatoid components. By applying this method, we showed that these components are found in different proportions in all tumors (E.score and S.score, respectively), leading to two opposite molecular gradients. These two gradients were related to histology and could fairly recapitulate existing molecular classifications (**Figure 1**). This observation indicates that the deconvolution approach offers a more standardized and finer-grained solution for describing tumor heterogeneity.

By combining transcriptome to methylome and miRNome analysis, we also specified the underlying oncogenic pathways driving the establishment of the epithelioid and sarcomatoid related cell entities. We highlighted the strong contribution of epigenetic regulation through DNA methylation

or miRNA expression deregulation, which are known to play a key role in mesothelial carcinogenesis together with chromosomal aberrations and genetic alterations [8]. We also observed a positive correlation of *NF2* and *TP53* mutations with the S.score, but contribution of genetic alterations deserved to be confirmed on larger series. Further functional studies are also needed to determine the exact contribution of the oncogenic pathways and to deepen the mechanisms of mesothelial carcinogenesis in the different cellular entities.

Another important point concerns the link between these histo-molecular gradients and the tumor microenvironment and the immune contexts. The S.score was positively correlated with infiltration of T cells and monocytes as well as fibroblasts and endothelial cells, while the E.score was correlated with natural killer cells infiltration and complement pathway, suggesting the involvement of the adaptive immune response in tumors with a high S-score and of the innate immune response in tumors with a high E-score. In addition, the S.score was strongly associated with high expression of most immune checkpoint inhibitors, including *PDL1* (*CD74*, best known as *PDL1*) and *CTLA4*, whereas only *TNFSF14* and *VISTA* overexpression was associated with the E.Score. Our results are consistent with the previously observed association of PDL1 protein expression with non-epithelioid MPM, poor clinical outcome, and increased lymphocyte infiltrates by immunohistochemistry [9]. The advantage of the S.score is to provide a finer overview into the immune landscape than immunohistochemistry.

The key input of our study was to reveal the potent clinical interest of histo-molecular gradients to characterize both intra and inter-tumor heterogeneity in MPM. We demonstrated that the S.score has a high prognostic value, superior than histologic and molecular classifications. In addition to the impact on prognosis, our results should also have an impact on personalized therapeutic strategies in MPM, particularly targeted therapies and immunotherapies. First, we highlighted that these histo-molecular gradients may guide therapeutic strategies such as targeted therapies by identifying, in preclinical studies, potent anti-cancer compounds such as Rho-associated protein kinase (ROCK) and WEE1 inhibitors whose efficacies were correlated with a high S-score in MPM. Second, the strong link between the S.score and T lymphocytes infiltration and immune checkpoint inhibitors expression supports that a high S.score could be predictive of immunotherapy based on anti-PDL1 and anti-CTLA4 inhibitors. This is particularly important given the recent promising results of this immunotherapy for some MPM patients [10]. The next crucial step is now to evaluate the correlation between the S.score and patients' response to immunotherapy in clinical trials.

Our study not only presents important new findings regarding MPM heterogeneity, but also fully reshapes the way in which we think about pathology and analyze tumor heterogeneity and biology using molecular data. We believe that our findings will greatly inspire researchers in the field of oncology.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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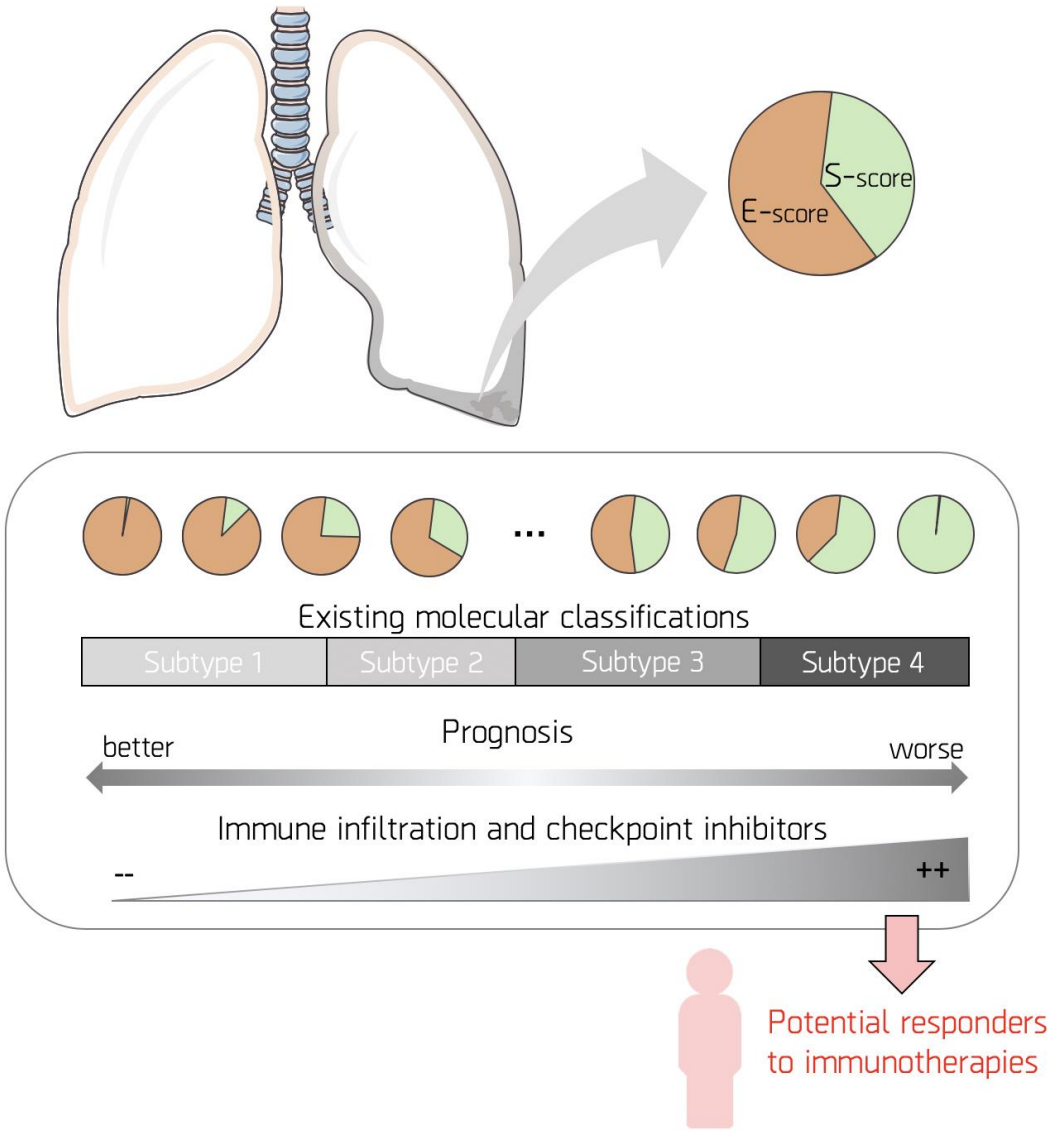
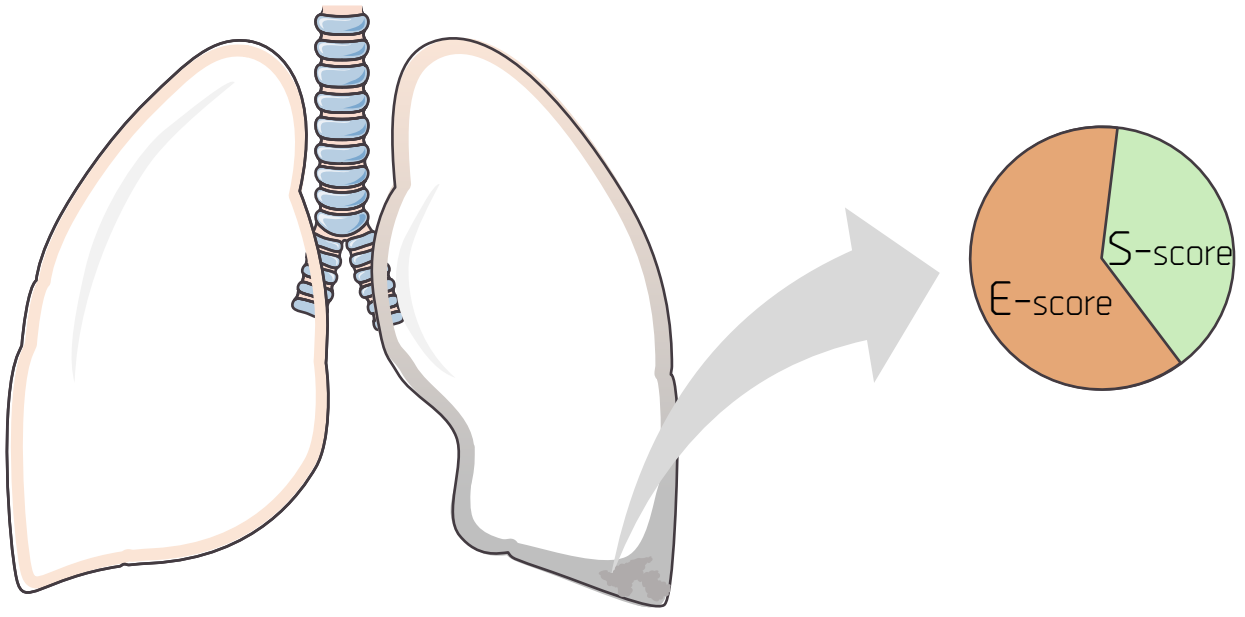
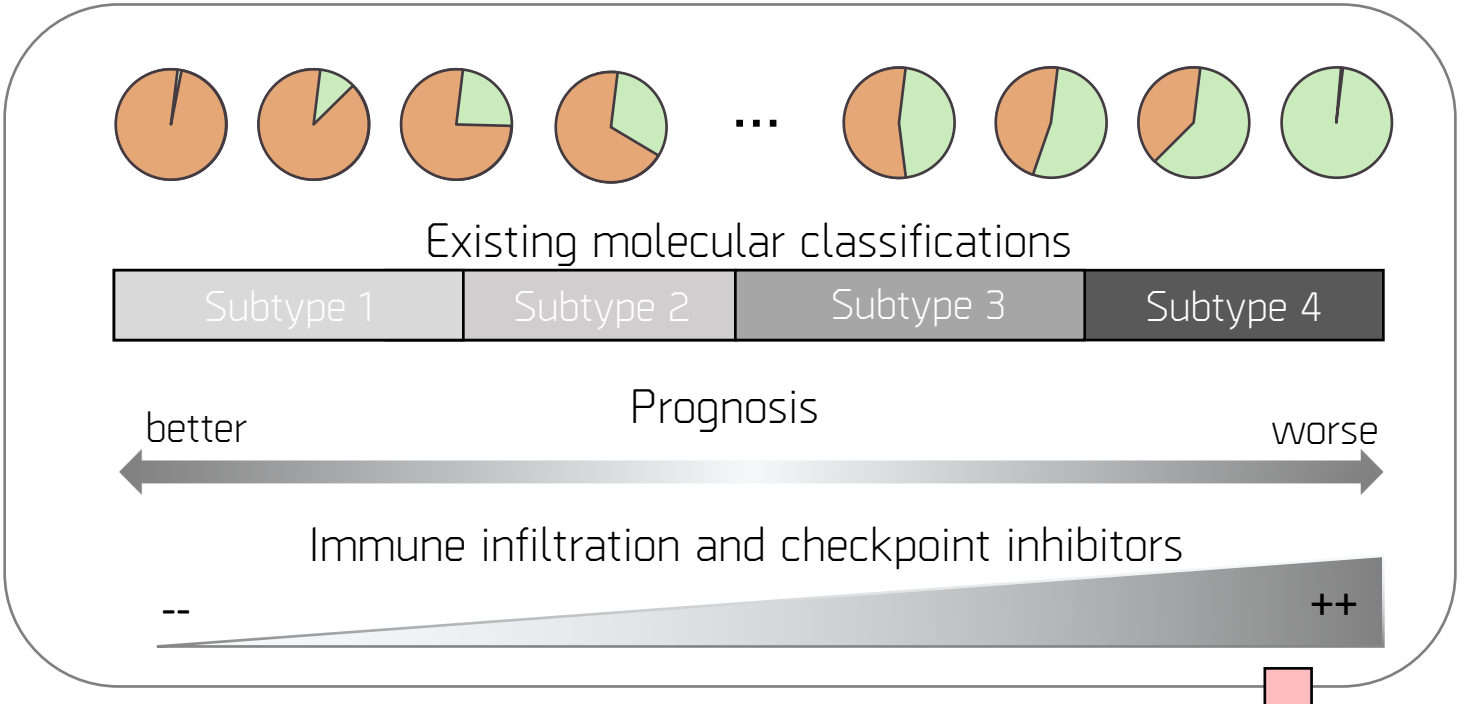


Figure 1. Dissecting malignant pleural mesothelioma (MPM) heterogeneity through a deconvolution approach. A MPM tumor can be decomposed into an epithelioid component and a sarcomatoid component in a certain proportion (E-score and S-score standing for epithelioid- and sarcomatoid-components proportion, respectively). These histo-molecular components recapitulate existing molecular classifications and are strongly associated with prognosis and the immune context. These findings may guide personalized therapeutic strategies in MPM in particular for immunotherapies.


E-score
S-score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Existing molecular classifications
Subtype 1
Subtype 2
Subtype 3
Subtype 4
Prognosis
better
worse
Immune infiltration and checkpoint inhibitors
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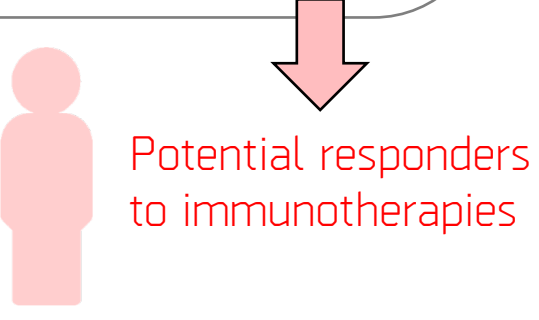
Potential responders
to immunotherapies