Early squamous cell lung carcinoma: prognostic biomarkers for the many

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When Aschoff published his landmark textbook Pathologische Anatomie in 1909,[1] lung carcinomas were barely mentioned. Their classification into histological subtypes was also very basic as they were simply described as being 'usually made up by cylindrical cells, rarely being medullary, colloid or cancroid'.[2] Since then a lot has changed because of our increased knowledge and, unfortunately, because of a dramatic increase of cases of lung carcinoma following the epidemic of cigarette smoking. Now we know that, under the umbrella of lung carcinomas, there are several different neoplastic diseases.[3] Squamous cell carcinomas (SCC) represent one of the major types of lung epithelial neoplasm, and three histological subtypes of invasive SCC are identified in the most recent WHO classification: keratinising, non-keratinising and basaloid[3] plus the preinvasive 'in situ' type.

Despite our advances in understanding the biology of these tumours, treatment for the largest group, the non-small cell lung carcinomas (NSCLC) of which SCC are part, it is still mainly guided by the tumour-node-metastasis (TNM) staging system, with surgery as first-line choice in early carcinomas (stages I, II and II according to the TNM system).[4] Although curative in a number of people, surgical treatment eventually still fails in a large number of cases. Only 67% of patients with Stage IA survive 5 years, while just 25% are still alive, at the same time, if the stage is IIIA.[5] The number of clinical trials evaluating the effect of adjuvant postoperative chemotherapy is therefore increasing to try to improve the outcome of surgery; however, the results are, so far, modest. When evaluating a patient for adjuvant treatment, the main predictive factor so far identified and currently used in clinical practice is still the TNM stage. Despite an increasing number of studies performed, no prognostic and/or predictive biomarkers have been yet recommended for patient management outside trials.[6]

The paper from Martinez-Terroba *et al*,[7] now published on *Thorax*, is not the first to address this problem. Yet, there is one crucial difference between this study and other investigations that have searched for biomarkers to improve management of early lung SCC. This study has been planned aiming not just to identify biomarkers as a such, but to find biomarkers that can be easily used, not only in basic research or trial setting but also in every day clinical practice. They achieved this by producing a 5-protein immunohistochemical signature, an easily reproducible technique. This proposed signature therefore can be easily adopted by any diagnostic histopathology laboratory. In doing so, the authors follow the methodological approach proposed by Subramanina and Simeon.[8] In their essay, the two authors draw attention to the fact that although there are by now in literature a great number of gene-expression prognostic signature reported, not many have been able to reach clinical practice. In their opinion, this could be due to several factors. One issue is the lack of clinical focus when the signature is investigated, for example, a signature for a specific histological subtype, while other problems are the scarcity of evaluation in independent data sets especially in prospective studies. They argue that this is mostly due to the fact that many of these signatures are very large and/or based on complex high throughput techniques which are well outside the reach of most clinicians. More user-friendly signatures should be designed so that can they can be easily and widely tested in large clinical trials or, second best, validated retrospectively on specimens from already concluded clinical studies.[8] By being easy and cheap to perform, such signatures would be amenable of successful transfer to every day clinical practice.

In tumours other than lung cancer, some easy to use immunohistochemical signatures, derived from larger high throughput molecular studies, have been developed. The most widely used in haematopathology is the one described by Hans *et al*.[9] By using a panel of three antibodies, against CD10, Bcl6 and Mum1, respectively, it provides an easy to use way to classify diffuse large B cell lymphoma (DLBCL) into germinal centre or non-germinal centre (activated) types in any clinical histopathology setting. This classification of DLBCL was originally described on a messenger RNA (mRNA) transcriptomic study published in 2000.[10] One group of lymphomas had an mRNA profile similar to that of normal germinal centre B cells ('germinal centre-like DLBCL'), while another type of expressed genes normally translated when peripheral blood B lymphocytes are activated 'in vitro' ('non-germinal centre' or 'activated B-like' DLBCL). Patients with germinal centre-like DLBCL have a significantly better overall survival than those with activated B-like DLBCL[10] and a better response to chemotherapy.[11] Therefore, the translation of this complex molecular signature into a three antibodies panel has been essential to allow its widespread use in clinical setting, leading to the possibility of easily entering patients into trials.[12]

Mantle cell lymphoma is another disease with a very heterogeneous outcome and response to treatment. A 17-gene mRNA signature has now been identified as a robust prognostic tool. Whether it can be used as predictive biomarker is still to be investigated.[13]

A comparable study in lung adenocarcinoma has identified a 10-gene mRNA signature providing a diagnostic tool for these tumours in their early stages.[14] Should these two signature be confirmed in their clinical utility, both would be a good basis to develop workable immunohistochemical diagnostic panels.

The approach of Martinez-Terroba and colleagues is not alternative to studies producing larger signatures but it is complementary. The authors have identified their genes through an extensive search and analysis of literature and available data base of high throughput gene expression studies. Keeping in mind the need to develop a practical signature that can be adopted by practising histopathologists, they have also taken in consideration some practical aspects, for example, it was not enough for a gene to be a good marker, the availability of a reliable antibody was also a determinant factor towards the selection of a biomarker. A second important issue for the practical application of this proposed 5-protein signature is its complementary role to the TNM staging, which is routinely performed all over the world.

The final aim of this quest is to be able treat the patients with a higher risk. To achieve this goal, it is desirable to have

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available also predictive markers. One example for NSCLC is immunotherapy with programmed cell death protein 1 (PD-1)/programmed cell death-ligand 1 (PD-L1) checkpoint inhibitors, which is now being tested both as first-line and second-line treatment.[15] These clinical studies require good predictive biomarkers, and immunohistochemistry for PD-L1 is currently being examined and will soon hopefully enter clinical use.[16]

Data from this study provide also some clues for further predictive biomarkers that could flank the one for immunotherapy. Two of the proposed biomarker are of particular interest, ribonucleotide reductase M2 subunit (RRM2) and glucose transporter 1 (GLUT1) (SLC2A1).

RRM2 inhibition by small interfering RNA causes cell cycle arrest and induction of apoptosis,[17] and compounds specifically designed to target RRM2 are now being investigated. One example is trans-4,4′-dihydroxystilbene (DHS). This molecule induces downregulation of RRM2 mediated by Cyclin F and as a consequence DNA replication is inhibited, the cell is arrested in S phase and, subsequently, goes in apoptosis following extensive DNA damage.[18]

The other proposed biomarker of interest, as far as predictive value is concerned, is GLUT1 (SLC2A1). GLUT1 is a major glucose transporter; it is present on endothelial cells of brain vessels, at the blood–brain barrier and is responsible for the entry of glucose into the central nervous system. In 1999, Lazar *et al*[19] demonstrated that abnormal high levels of GLUT1 can be present on both adenoma and carcinoma of the thyroid. Subsequently, excessive expression of GLUT1 have been found in many types of tumours. These neoplasms with higher GLUT1 presence have usually a poorer prognosis than those with lower levels.[20] A worst outcome also for patients expressing high levels of GLUT1 in SCC has been reported by the present study. GLUT1 is one of the 14 glucose transporters belonging to the GLUT family.[21] Glucose is critical for providing energy to the cancer cells and therefore glucose transporters are now under scrutiny as emerging targets for treatment. The rationale is that, when starved of glucose, the cell has an increased activation of the tumour suppressor LKB1-AMP-kinase pathway[21] leading to cell cycle arrest and apoptosis. Several chemical compounds and anti-GLUT1 antibodies are currently under investigation. They act in different way: some decrease the levels of mRNA coding for GLUT1, other inhibits its transport function while some decreases the levels of protein.[21]

In conclusion, the availability of easy to use signatures with prognostic and, possibly, predictive value is going to be determinant to improve our ability to offer treatments increasingly accurate to oncological patients and the present study is another step forward.

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