EDITORIAL

Role of Molecular Biology in Histopathology

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Pathology has evolved significantly in the past 50 years. Even I can feel lot of changes in last 10 years of my histopathology practice starting from my residency days till the present day and things are now developing even at a greater pace. It is very difficult to keep up the pace with these ever-changing and developing diagnostic modalities.

Light microscopy was the sole examination technique at first and pathology was comparatively simple. However, with increasing tumor diagnosis and tumor burden across the globe, a strong need was felt to strengthen the classification systems so that diseases could be put into specific categories and hence treated in a more specified and accurate manner. This need gave birth to the technique of immunohistochemistry, which was based on the principle of antigen retrieval and binding of specific antibodies designed for a particular antigen. Nowadays well-developed histopathology departments use hundreds of antibodies in order to diagnose different kinds of diseases particularly several kinds of neoplastic diseases including benign and malignant tumors. We can very confidently classify tumors broadly into categories of carcinoma, lymphoma, sarcoma, neuroendocrine tumor, melanoma, perivascular epithelioid cell tumor, metastatic tumor etc. Each of these broad groups can be further classified into specific categories based on histological and immunohistochemical features. This has revolutionized the treatment options for patients. However, every technique has a limitation. After immense and colossal research in the field of immunohistochemistry, it is now very much known that immunohistochemistry has got its own limitations.

Despite, for example, cytokeratins which are meant to stain carcinomas can also be expressed in sarcomas. Likewise, melanocytic markers which are known to diagnose melanoma can also show positivity in perivascular epithelioid cell tumors. CD99 initially considered a very specific marker for Ewing's sarcoma is now known to show expression in osteosarcoma (small cell variant), lymphoblastic lymphoma and synovial sarcoma. CD56 also shows its positivity in a number of tumors including carcinomas, lymphomas, and sarcomas. Therefore, we cannot overemphasize the fact that a single technique is not enough to diagnose a particular disease. It is the combination of clinical features, radiological features, histological findings and immunohistochemical results that is important for an accurate diagnosis.

Researchers long ago started feeling that even classifying tumors based on histology and immunohistochemistry was not enough. The reason was the observation that patients having same age, gender, type, and stage of disease behaved differently. For instance, patients having lung adenocarcinoma, breast carcinoma, malignant melanoma, colorectal adenocarcinoma, thyroid carcinoma, sarcomas, gastrointestinal stromal tumor (GIST), ovarian carcinoma, lymphoma behaved very much differently even if they belonged to the same group of disease and shared similar demographic details and same type and stage of the disease. These findings led and compelled investigation, exploration, analysis, testing, and experimentation of genetics of disease. I can assure you that in future genetics will be the part and parcel of every field related to diagnostics, especially pathology.

At this point of time, fluorescence in situ hybridization (FISH), PCR, next-generation sequencing (NGS) and tissue microarrays are the genetic techniques used in combination with histology and immunohistochemistry for diagnosing and classifying malignancies. Now it is established that malignancies having similar histological

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and immunohistochemical features and belonging to the same group can behave differently on account of their different genetic phenotypes. Using this fact, scientists are working very hard to develop targeted therapies against different types of genetic aberrations e.g. identification of many oncogenes and tumor suppressor genes.

NGS has particularly helped a lot in understanding the genetics of a large number of diseases and malignancies as it has the capability of sequencing and analyzing millions of DNA molecules at reduced rates. Some of the genetic aberrations are of prognostic and other are of therapeutic interest. With the help of NGS, mutations of interest are studied for their prognostic and therapeutic values and few are selected to develop targeted therapies. NGS is not available routinely in Pakistan and very rarely used for research purposes at very few centers. I would like to give you few examples of the role of genetic studies in finding out molecules of treatment and prognostic significance. Lung adenocarcinomas can look alike histologically but have different genetic makeups. Few genes of interest in lung adenocarcinoma are EGFR, ALK and ROS1 against which targeted therapies have been developed e.g. erlotinib against EGFR mutation and crizotinib in ALK and ROS1 mutated adenocarcinomas. GIST having KIT and PDGFRA mutations can benefit from imatinib or sunitinib therapy whereas SDH deficient GISTs do not benefit from this therapy and require second line drug regime. Colorectal carcinomas having KRAS mutations behave and respond to therapy differently than colorectal carcinomas having microsatellite instability (MSI). Breast carcinomas which are ER and PR positive are given different therapy than those which are triple negative for ER, PR and HER-2/neu or which are only HER-2/neu positive. These are very few basic examples of very common tumors like a drop in the ocean. Unfortunately, the discovery of genetic aberrations has outpaced the development of targeted therapies.

PCR technique is cheaper and very much simpler than NGS. The particular gene of interest can be tested in tumor tissue and treatment can be based on the results. Of course, only limited number of genes can be studied as compared to NGS in which millions of molecules can be assessed. The ability of the molecular department to run PCR tests on formalin fixed tumor tissue is a big advantage for the pathologists and treating oncologists and every effort should be made to acquire this expertise.

FISH technique is another very useful and very simple technique which works on the principle of binding of the fluorescent probe to a complementary antigen of interest. We use quite a few FISH tests in our department. We use HER-2/neu amplification studies in breast carcinomas, FISH break apart probes for Ewing' sarcoma (EWSR1 gene rearrangement), synovial sarcoma (X;18) and alveolar rhabdomyosarcoma (FOXO1 gene rearrangement), N-myc and MDM2 amplifications studies for neuroblastoma and liposarcoma respectively, break apart probe for ALK gene rearrangement in lung adenocarcinoma and 1p and 19q co-deletion studies in oligodendrogliomas. Some of these results have therapeutic and some have prognostic value for the patient.

Every institute can tailor these techniques according to their own needs. Pathologists should assess the tumor burden and tumor types being diagnosed in their institutes and discuss the need to develop different molecular techniques with oncologists and molecular biologists. This not only helps to diagnose tumors with accuracy but also help oncologists to assess prognosis and treatment plan of different tumors.

I think we have entered the era in our lives where genetics has gained an utmost importance in diagnostics and therapeutics of diseases and nobody can deny the importance of genetics. Therefore, in spite of avoiding the understanding of genetics, every university and college should make genetics a compulsory part of their curriculum as it has already been included in the curriculum of undergraduate and postgraduate students in the developed world so that doctors have the basic knowledge of genetic techniques by the time they become qualified pathologists.