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Therapeutic Pathology- The Pathology of Tomorrow

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The Nomenclature- What's in the Name!

As pure science, pathology has always been the backbone of every aspect of diagnosis in medical practice. Although the main focus has been on the classification of the disease entities and their association with the prognosis of the condition, the focus has been shifting towards more practical applications- the 'therapeutic uses'.

Therapeutic pathology or *Predictive pathology*, as this application of pathology can be rightly termed, with its integrated molecular categorization of the disease condition will be the most awaited futuristic therapeutic application of technology in the medical field. This technique will have specific "individualized" therapeutic approaches using the analysis of a single or only a few parameters that will help clinicians and patients decide on the existing treatment modalities [1]. Currently, this approach is mostly based on classical histopathology combined with immunohistochemistry and specific biomarkers, and has been proven highly successful in the management of a good number of conditions [2]. Alternatively, the name '*Personalised Pathology*' can be used as the results of the diagnostic services are directly related to individual or personalized therapy applications.

Personalized therapy demands a much greater understanding of the disease biology ranging from morphological to epigenetic grounds. More challenges await during the drug design and the clinical trials of the drug. Unsurprisingly, a close collaboration among research institutions, regulatory authorities, and pharmaceutical companies is required to fulfil this personalized treatment science. For the above-mentioned coordination that is necessary for its fullest applications, this branch of pathology can also be termed as *'Companion diagnostics'* [1].

The Journey of Advancement- The Journey Matters More than Destiny!

It all began with the innocent and initial quest to know more and more details of genetic changes in some of the rare conditions with uniform biological and clinical behavior. These studies led to rapidly translatable discoveries in diagnostics and tumor taxonomies, as well as providing insights into cancer biology. The initial studies with comparative genomic hybridization or CGH revealed repetitive patterns of DNA imbalances in many tumor types [2- 4]. These genetic variations were so unique for the disease under study, that encouraged researchers to study similar genetic variations in other disease conditions.

The Pathology Pathway- popularly as it is called, intends to integrate genetic, molecular, and biological information into disease classification systems and treatment. Thus, making this huge amount of data available for medical doctors and scientists to grasp and apply for the benefit of individual patients [5]. This system has three fundamental components, i.e.,

(1) The cellular context of the disease;

(2) The genes and proteins being affected, i.e., the molecular pathways;

(3) The cellular mechanisms being altered, i.e., the cellular pathways

In each of the above components, multiple methods of diagnostic techniques were tried and experimented with to prove the already known mechanisms and pathogenesis including the known mutations. Approach through *'the pathology pathway'* paved the way to verify the innovative

techniques in biotechnology and their application in diagnostic pathology for its utilization in '*therapeutic pathology*'.

Molecular Diagnostic Techniques- A Personalized Approach to Healthcare!

In this fascinating field of innovation, techniques, and technology are added day in and day out. A few of the useful, available, and approved methods are briefed here-

Polymerase chain reaction (PCR)

Technique: PCR is the most frequently used molecular technique in a molecular pathology laboratory. This technique uses a pair of priming complementary sequences (oligonucleotide primers) flanking a location of interest, together with unique heat-resistant polymerases (DNA copying enzymes), multiple copies of a targeted chimeric gene can be obtained. PCR can also be used to amplify an RNA target sequence; the procedure is termed reverse transcriptase PCR (RT-PCR). The recent development of "real-time" PCR (Q-PCR) allows for the real-time quantitation of PCR products following each of the 40 amplification cycles thereby offering a great rapid quantitative advantage.

Utility: PCR has a wide range of applications, while the Q-PCR is of great utility in the assessment of minimal residual disease following novel targeted therapy against specific molecular defects as well as bone marrow transplantation for myelogenous leukemia [6-8].

Fluorescent in situ hybridization (FISH)

Technique: This technique uses fluorescence-labelled oligonucleotide probes that specifically attach to their complementary DNA sequence target on the genome and label that region with fluorescence color that can then be easily visualized under a fluorescence microscope. Added advantages of FISH include that it can be performed on cells in the dividing (metaphase) as well as resting (interphase) stages and can very well be performed on fresh frozen as well as archival cytological smears or paraffin-embedded tissue sections.

Utility: Currently, FISH is often used in the evaluation of HER2/neu oncogene amplification in breast carcinoma and for the detection of different translocations in chronic myelogenous leukemia and acute myelogenous leukemia, and to identify chromosomal translocations [9]

Spectral karyotype imaging (SKI)

Technique: SKI is based on the use of 23 sets of chromosome-specific "painting" probes. Every probe is labelled with varying proportions of 3 fluorescent dyes. This allows each chromosome pair to be labelled by light of unique spectral emission. The pathologist can then readily identify any numerical chromosomal abnormalities (aneusomy) or any shifting in colored chromosomal portions (translocations).

Utility: The applications are similar to that of FISH.

DNA microarray and DNA chip technology

Technique: This technique undertakes gene expression profiling using DNA microarrays and holds great promise for the future of molecular diagnostics, allowing, in one assay, for simultaneous assessment of the expression rate of thousands of genes in a single sample. The 2 types of DNA microarrays that are widely used are cDNA microarrays and oligonucleotide/DNA chips.

Utility: BCR/ABL1 fusion in chronic myelogenous leukemia and similar pathologies.

Next generation sequencing (NGS)

Technique: This technique offers the ability to simultaneously sequence thousands to millions of relatively short nucleic acid sequences in parallel. Thus, generating more information at low costs when compared with large genome regions being sequenced. NGS promises multiple new diagnostic opportunities for researchers, molecular pathologists, and their patients as much of the genome is already available for technical interrogation. NGS can be limited to a pre-specified group of genes (targeted gene panels), can focus on the coding regions of all of the base pairs of the genome (whole exome sequencing (WES)), or can involve the analysis of the entire tumor genome, including the intronic regions (whole genome sequencing (WGS)) [10].

Application of artificial intelligence (AI)

With a huge amount of data pouring in from the rapidly exploring research, the role of artificial intelligence cannot be underestimated!

Challenges to Therapeutic Pathology- Making Way for Newer Advancements!

'Where there is a will, there is a way' holds true universally. With a large round of applause to the high-soaring achievements of molecular techniques, challenges come as a by-product, paving the way for betterment. Some challenges are addressed below-

The complex molecular changes, intra-tumoral heterogeneity of tumor cells, and stroma of the tumor

Cancers are mostly clonal proliferations however, the evolution of subpopulations of tumor cells with heterogeneity of molecular changes as well as the abundant stromal tissue can create difficulty in analytical methods.

An increasing number of druggable mutations

Genetic alterations in the form of multiple mutations are ever occurring during carcinogenesis. These mutations have an important role in therapeutic diagnostics as they help the application of targeted drugs during treatment. However, with the increasing number of mutations, personalized therapies pose a huge challenge for conventional study designs to apply for approval by the FDA or EMA [11] (**Table 1**).

Specimen-related challenges

The specimen undergoes a battery of processing which includes formalin-fixation, and paraffin-embedding that can alter nucleic acids, and not to neglect, intra-tumoral heterogeneity in space or time, the low tumor content in tumor samples can also decrease test sensitivity or lead to false-positive mutation calls [12-16]. To ameliorate this issue, an emerging field tests circulating tumor-specific markers like circulating tumor cells (CTC) or circulating tumor DNA (ctDNA), as well as RNAs, proteins, or metabolites, that are present in body fluids. Being minimally invasive these procedures can be repeated for tumor-specific diagnostic, prognostic, or predictive biomarkers [17]. Another advantage it tackles is intra-tumoral heterogeneity which is not expected in circulating tumor cells [18,19]

This version of pathology, 'Precision Medicine' as can be called, has gained access in daily practice and is ever challenging for molecular biologists and pathologists to provide relevant diagnostic information in the shortest time possible. Not surprisingly, not far off in time, the major role of laboratory diagnosis will be DNA and RNA analysis [2].

The Future is Hidden in the Signatures!

With on-going search and research to find the greatest possible details, various signatures have emerged. While discreet driver mutations associated with therapeutic targets or of diagnostic or prognostic value have seen therapeutic uses, the genomic profiles (*signatures*) are similar patterns of gene expression or inherited or somatic mutations across multiple genes or genomic regions. These tools called the ***'mutational signature'*** help group patients into various categories for response, outcomes, or other clinical features. Currently, such profiles are reported in numerous cancer types including hepatocellular carcinoma, breast cancer, brain cancer, and diffuse large B cell lymphoma [20-22].

Table 1. FDA and EMA approved biomarker matching targeted drugs and routine molecular pathology testing [13].

Gene/protein	Anticancer agent	Indications	Biomarker	Routine testing
Androgen receptor (AR)	Abiraterone, enzalutamide, dalurotamide, apalutamide	Prostate cancer	AR expression	IHC
PI3K (alpha and delta)	Copanlisib	Follicular lymphoma	PI3K mutation	DNA sequencing
ALK	Crizotinib, ceritinib, alectinib, lorlatinib, brigatinib	NSCLC	ALK translocation	FISH, IHC
BCL-2	Venetoclax	Chronic myeloid leukemia	BCL-2 protein expression, BCL-2 amplification/ translocation	IHC, FISH
BCR/ABL	Imatinib, dasatinib, nilotinib, bosutinib, ponatinib	Chronic myeloid leukemia	BCR/ABL1 fusion	IHC (FISH, DNA/RNA sequencing), PCR1
C-KIT, PDGFR	Imatinib	Gastrointestinal stromal tumor	c-KIT Exon 9 and 11 mutations, PDGFR mutations	IHC, DNA sequencing
FGFR2/3	Erdafitinib	Bladder cancer	FGFR3 mutations, FGFR2/3 fusions	DNA sequencing, FISH
PI3KCA	Alpelisib	Breast cancer	PI3KCA mutation	DNA sequencing
RAS (negative predictor)	Cetuximab, panitumumab	Colorectal cancer	KRAS/NRAS wildtype	DNA sequencing
PDGFRB	Imatinib	Myelodysplastic/ myeloproliferative syndromes	PDGFRB rearrangement	FISH

AR: Androgen Receptor; ER: Estrogen Receptor; FISH: Fluorescence In situ Hybridization; IHC: Immunohistochemistry; NSCLC: Non-small Cell Lung Cancer; PR: Progesterone Receptor

With constant improvement in clinician and patient education and the development of easily accessible online genomic knowledge banks will provide resources to aid data interpretation and clinical decision-making. As an example, the Precision Oncology Decision Support (PODS) platform provides clinical decision support for oncologists with information on genotype-matched therapeutics (including clinical trials) that are relevant to their patients [23-25].

Conclusion

According to the description in Mahabharata (Adi Parva/ Sambhava Parva), the Kauravas were created by splitting the single embryo into 100 parts and growing each part in a separate Kund or container. Genetics and technology will continue to evolve. The results of these developments depend on how responsibly we behave and apply them for the larger welfare of mankind.

Abbreviations

FDA: The United States Food and Drug Administration; EMA: The European Medicines Agency; IHC: Immunohistochemistry

References

1. Dietel M, Jöhrens K, Laffert MV, Hummel M, Bläker H, Pfitzner BM, et al. A 2015 update on predictive molecular pathology and its role in targeted cancer therapy: A review focussing on clinical relevance. Cancer Gene Therapy. 2015;22(9):417-30.

2. Bockmühl U, Schwendel A, Dietel M, Petersen I. Distinct patterns of chromosomal alterations in high- and low-grade head and neck squamous cell carcinomas. Cancer Res. 1996;56:5325-9.

3. Petersen I, Petersen S. Towards a Genetic-Based Classification of Human Lung Cancer. Anal Cell Pathol. 2001;22:111-21.

4. Ried T, Petersen I, Holtgreve-Grez H, Speicher M, Schröck E, Du Manoir S, et al. Mapping of multiple DNA gains and losses in primary small cell lung carcinomas by comparative genomic hybridization. Cancer Res. 1994;54:1801-6.

5. Petersen I. Classification and Treatment of Diseases in the Age of Genome Medicine Based on Pathway Pathology. International Journal of Molecular Sciences. 2021 Aug 30;22(17):9418.

6. Collins FS, McKusick VA. Implications of the human genome project for medical science. JAMA 2001;285:540-4

7. Diverio D, Rossi V, Avvisati G, De Santis S, Pistilli A, Pane F, et al. Early detection of relapse by prospective reverse transcriptase-polymerase chain reaction analysis of the PML/RARα fusion gene in patients with acute promyelocytic leukemia enrolled in the GIMEMA-AIEOP multicenter "AIDA" trial. Blood 1998;92:784-9.

8. Cazzaniga G, d'Aniello E, Corral L, Biondi A. Results of minimal residual disease (MRD) evaluation and MRD-based treatment stratification in childhood ALL. Best Pract Res Clin Haematol 2002;15:623-38.

9. Cazzaniga G, Lanciotti M, Rossi V, Di Martino D, Arico M, Valsecchi MG, et al. Prospective molecular monitoring of BCR/ABL transcript in children with Ph+ acute lymphoblastic leukemia unravels differences in treatment response. Br J Haematol 2002;119: 445-53.

10. Yip S, Christofides A, Banerji S, Downes MR, Izevbaye I, Lo B, et al. A Canadian guideline on the use of next-generation sequencing in oncology. Curr Oncol. 2019;26:e241–54.

11. Schrijver I, Aziz N, Farkas DH, Furtado M, Gonzalez AF, Greiner TC, et al. Opportunities and challenges associated with clinical diagnostic genome sequencing: a report of the Association for Molecular Pathology. The Journal of Molecular Diagnostics. 2012 Nov 1;14(6):525-40.

12. Moorcraft SY, Gonzalez D, Walker BA. Understanding next generation sequencing in oncology: a guide for oncologists. Crit Rev Oncol Hematol. 2015;96:463-74.

13. Malone ER, Oliva MB, Sabatini PJ, Stockley TL, Siu LL. Molecular profiling for precision cancer therapies. Genome Medicine. 2020 Jan 14;12(1):8.

14. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366:883-92.

15. Bedard PL, Hansen AR, Ratain MJ, Siu LL. Tumour heterogeneity in the clinic. Nature. 2013;501:355-64.

16. Lee CK, Kim S, Lee JS, Lee JE, Kim SM, Yang IS, et al. Next-generation sequencing reveals novel resistance mechanisms and molecular heterogeneity in EGFR-mutant non-small cell lung cancer with acquired resistance to EGFR-TKIs. Lung Cancer. 2017;113:106-114.

17. Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet. 2019;20:71-88.

18. De Mattos-Arruda L, Mayor R, Ng CKY, Weigelt B, Martinez-Ricarte F, Torrejon D, et al. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. Nat Commun. 2015;6:8839.

19. Dietz S, Schirmer U, Merce C, von Bubnoff N, Dahl E, Meister M, et al. Low input whole-exome sequencing to determine the representation of the tumor exome in circulating DNA of non-small cell lung cancer patients. PLoS One. 2016;11:e0161012.

20. Chapuy B, Stewart C, Dunford AJ, Kim J, Kamburov A, Redd RA, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. Nat Med. 2018;24:679-90.

21. Davies H, Glodzik D, Morganella S, Yates LR, Staaf J, Zou X, et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. Nat Med. 2017;23:517-25.

22. Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J,Rebouissou S, et al. Exome sequencing of hepatocellular carcinomas

identifies new mutational signatures and potential therapeutic targets. Nat Genet. 2015;47:505-11.

23. Rossi G, Ignatiadis M. Promises and pitfalls of using liquid biopsy for precision medicine. Cancer Res. 2019;79:2798-804.

24. Scott SN, Ostrovnaya I, Lin CM, Bouvier N, Bochner BH, Iyer G, et al. Next-generation sequencing of urine specimens: a novel platform for genomic analysis in patients with non-muscle-invasive urothelial carcinoma treated with bacilli Calmette-Guerin. Cancer Cytopathol.2017;125:416-26.

25. Hickmann AK, Frick M, Hadaschik D, Battke F, Bittl M, Ganslandt O, et al. Molecular tumor analysis and liquid biopsy: a feasibility investigation analyzing circulating tumor DNA in patients with central nervous system lymphomas. BMC Cancer. 2019;19:192.