Detection of clinically relevant abnormalities in genes

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- There are two broadly related areas of clinical practice that rely on molecular analysis. First, analysis of tumour DNA may improve diagnostic precision, enhance treatment plans and help predict clinical outcome. Second, it may suggest or detect germline mutations that are characteristic of an inherited disease. This can confirm non-neoplastic conditions, such as cystic fibrosis, or be used to diagnose a hereditary predisposi - tion to cancer, e.g. Lynch syndrome. - Mutational analysis requires extraction of DNA from tis - sue (or from other sources such as blood) and often includes sequencing-based screening methods (e.g. Sanger sequencing, pyrosequencing) (Figure 11.29) , screening methods compar - ing mutated with normal DNA and targeted m utation detec - tion methods. NGS (Figure 11.30) emerged relatively recently . The term NGS encompasses several platforms, each of which performs - massively parallel sequencing, allowing simultaneous examina - tion of millions of fragments of DNA for molecular alterations. It is applicable to formalin-fixed tissue, allows evaluation of many DNA regions in a single assay and displays increased analytical sensitivity (i.e . the ability to detect low-frequency alleles) compared with Sanger sequencing or conventional PCR. Widely clinically used targeted NGS panels can identify - multiple known mutations and other variants in 20–500 genes of interest in a single test. New powerful platforms can detect not only point muta - , RB), tions but also copy number variants and gene fusions in more than 100 genes involved in human oncogenesis with minimal nuclear acid (DNA and RNA) sample input. Adequate amounts of good quality tumour DNA are nec - essary for the success of these techniques. Histolog y samples usually include both non-neoplastic tissue and tumour. The pathologist plays a crucial role in assessing the suitability of - tissue samples for molecular analysis by analysing tumour cell content as a percentage of all cells, cellularity and degree of necrosis. Microdissection of the area of interest using conven - tional techniques or laser-assisted approaches improves yields of tumour-derived DNA. Summary box 11.13 Genes and carcinogenesis /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF Indications for molecular analysis of tumour tissue /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF Summary box 11.15 Detection methods for main molecular changes /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF

(b) Figure 11.30 Immunohistochemical screening for mismatch repair gene abnormalities in a carcinoma. (a) There is retention of nuclear MLH1 expression (arrows showing positively staining brown neoplas tic nuclei). (b) In contrast, there is loss of MSH2 expression (no staining in neoplastic nuclei), suggesting a mismatch r epair gene abnormality. Genes (Proto-) oncogenes KRAS BRAF EGFR BCL2 Tumour suppressor genes TP53 BRCA1/2 Pathways Proliferation and signal transduction Cell cycle control DNA repair Apoptosis Diagnosis and classi /f_i cation Selection of therapy Prognosis Staging Monitoring disease burden Screening for germline mutations Con /f_i rmation of neoplasia (e.g. clonality) Point mutations and small insertions and deletions: NGS, PCR Fusions: FISH, NGS, PCR Ampli /f_i cations: FISH, NGS Tumour mutation burden: NGS Immunohistochemistry may be a very useful initial test, and is often suf /f_i cient

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