SEQUENOM®

Mutation profiling in tumor samples using the Sequenom OncoCarta™ Panel

Sequenom's MassARRAY® system enables sensitive and rapid somatic mutation profiling in solid tumors or cell lines. Our validation studies revealed mutations in most of the oncogenes analyzed, and the mutation frequency was consistent with previous reports. We also found new low-frequency mutations in our tumor samples, indicating that rare and potentially targetable mutations can be identified with this approach.

Accurate diagnosis and tumor classification is of fundamental importance both as a prognostic indicator and to determine most effective treatment modality. Although the current basis of tumor taxonomy is grade, stage and type, in conjunction with other histopathological indices, tumors can be more accurately characterized by their molecular profile. Rare mutations present in only a small percentage of the tumor mass may be important in the natural selection process that takes place during tumorigenesis or once an individual undergoes treatment. Thus, a priori knowledge of the presence of a clinically relevant mutation can provide important information by which to classify the tumor and to select rational intervention. The aims of profiling cancer mutations are to provide a molecular snapshot of the mutations in each individual tumor sample for understanding the pathways involved in driving the cancer's growth and, ultimately, to tailor the best therapeutic strategy to each patient. The cost, complexity and relative lack of sensitivity in identifying mutations that contribute to each cancer have hampered the development of translational medicine strategies. However, several recent studies have been described using Sequenom's primer extension and MassARRAY MALDI-TOF mass spectrometry–based methods to resolve many of these issues[1–3].

Oncogene mutations do not usually occur randomly but are more frequent in certain genomic regions. Sequenom has developed an oncogene panel based on the mutations analyzed in ref. 1, where the researchers analyzed the frequency and distribution of known mutations in different tumor types. The OncoCarta™ Panel v1.0 offers rapid, parallel analysis of 238 simple and complex mutations across 19 common oncogenes. The panel consists of a set of predesigned and prevalidated assays for sensitive and efficient mutation screening of tumor samples for research purposes only. The OncoCarta Panel consists of 24 wells per sample and requires less than 500 nanograms of DNA to analyze all 238 mutations (**Fig. 1**).

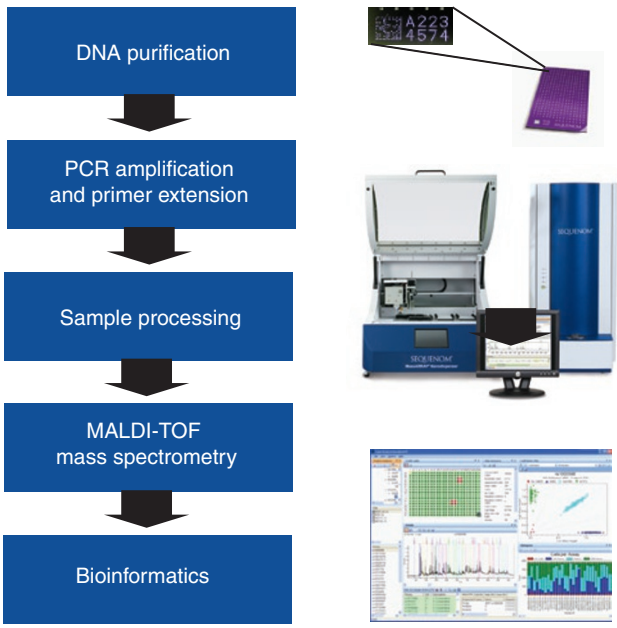

Figure 1 | Workflow of oncogene mutation profiling with the Sequenom MassARRAY system and OncoCarta Panel.

Assay design and data analysis

The predesigned content in the OncoCarta Panel v1.0 eliminates the time required to design assays. Comprehensive analysis software reduces downstream data analysis. The OncoCarta Panel has several new features and improvements, including robust TypePLEX® chemistry with single-base extension for higher specificity, and new SpectroCHIP® II solid surface supports for decreased likelihood of adduct formation and higher signal-to-noise ratio. Assays

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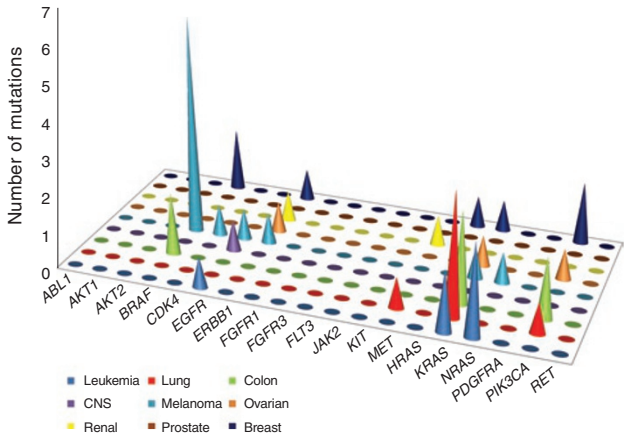

Figure 2 | Oncogene mutations across tumor types.

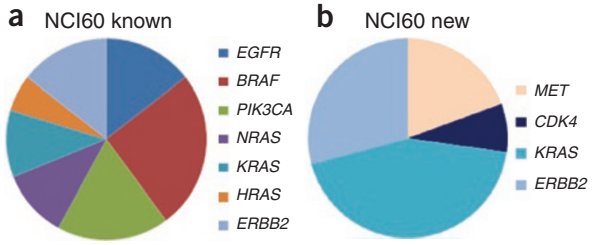

Figure 3 | Total known (**a**) and new (**b**) mutations in NCI60 DNA found using the OncoCarta v1.0 Panel.

are designed and optimized to ensure detection of low-frequency mutations. Complex mutations are resolved over multiple wells for increased accuracy in mutation frequency call rates.

OncoCarta data analysis is performed using Sequenom's analysis software, Typer Analyzer. The software includes customizable parameters that can be adjusted on the basis of the desired probabilities, including desired mutation frequency. The software also provides default settings, which are the suggested parameters from validation experiments. An automated function allows creation of mutation frequency reports.

Formalin-fixed, paraffin-embedded tissue

To validate the OncoCarta Panel, we used two biologically relevant model systems. The first was a set of 96 paraffin-embedded cancer samples. For these samples, 74 mutations had been previously identified using conventional dideoxy sequencing. The OncoCarta Panel identified 70 out of the 74 mutations. The OncoCarta Panel also revealed the presence of previously unknown mutations. We identified a total of 31 new mutations using the OncoCarta Panel, so that more than 40% of mutations detected were new, with frequencies of occurrence ranging from 10%–40%.

We mapped the distribution of mutations across tumor types (**Fig. 2**). Notably, we observed many *BRAF* mutations as well as *CDK4*, *EGFR*, *ERBB2*, *KRAS* and *NRAS* mutations in the melanoma samples. However, for colon cancer tissue, we observed a few mutations in *BRAF*, *KRAS* and *PIK3CA*, whereas leukemia samples had *EGFR*, *KRAS* and *NRAS* mutations. Taken together, these results show that the OncoCarta Panel can be used to detect known and unknown mutations as well as co-occurring mutations.

NCI60 cell line DNA

In the second model system, we used DNA from 60 cell lines (NCI60 panel) for mutation detection (**Fig. 3**). The NCI60 cells are thoroughly characterized using gene expression, DNA methylation and karyotype information, and have also been sequenced by the Wellcome Trust Sanger Institute. The OncoCarta Panel contains a subset of 37 mutations within this particular sample set. For more than 86% of these same mutations, the data from OncoCarta Panel were in concordance with the COSMIC database (http://www.sanger.ac.uk/genetics/CGP/cosmic/). In addition, the OncoCarta Panel detected seven previously unknown mutations, resulting in a 20% increase in detected mutations. The newly identified mutations ranged in frequency between 10% and 50%, and they may not have been as easily discernable with standard Sanger sequencing methods.

Summary

Sequenom's OncoCarta Panel was validated using samples with known mutations and was used to identify and quantify previously unreported mutations. By design, the panel covers a large number of oncogene regions that would normally require at least 60 sequencing runs per sample. The panel provides a comprehensive set of predesigned assays to simultaneously investigate not only commonly occurring mutations such as those in *EGFR* or *KRAS*, but also infrequent mutations in the population that could be relevant to cancer progression or disease outcome.

The OncoCarta Panel v1.0 is for research use only, not for use in *in vitro* diagnostics.

1. Thomas, R.K. *et al.* High-throughput oncogene mutation profiling in human cancer. *Nat. Genet.* **39**, 283–284 (2007).
2. Jurinke, C. *et al.* MALDI-TOF mass spectrometry: a versatile tool for high-performance DNA analysis. *Mol. Biotechnol.* **26**, 147–163 (2004).
3. Vivante, A. *et al.* High-throughput, sensitive and quantitative assay for the detection of BCR-ABL kinase domain mutations. *Leukemia* **21**, 1318–1321 (2007).