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Review

Review of sequencing platforms and their applications in phaeochromocytoma and paragangliomas

Suja Pillai, Vinod Gopalan, Alfred King-Yin Lam*

Cancer Molecular Pathology, School of Medicine and Menzies Health Institute Queensland, Griffith University, Gold Coast, Q4222, Australia

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ABSTRACT

Genetic testing is recommended for patients with phaeochromocytoma (PCC) and paraganglioma (PGL) because of their genetic heterogeneity and heritability. Due to the large number of susceptibility genes associated with PCC/PGL, next-generation sequencing (NGS) technology is ideally suited for carrying out genetic screening of these individuals. New generations of DNA sequencing technologies facilitate the development of comprehensive genetic testing in PCC/PGL at a lower cost. Whole-exome sequencing and targeted NGS are the preferred methods for screening of PCC/PGL, both having precise mutation detection methods and low costs. RNA sequencing and DNA methylation studies using NGS technology in PCC/PGL can be adopted to act as diagnostic or prognostic biomarkers as well as in planning targeted epigenetic treatment of patients with PCC/PGL. The designs of NGS having a high depth of coverage and robust analytical pipelines can lead to the successful detection of a wide range of genomic defects in PCC/PGL. Nevertheless, the major challenges of this technology must be addressed before it has practical applications in the clinical diagnostics to fulfill the goal of personalized medicine in PCC/PGL. In future, novel approaches of sequencing, such as third and fourth generation sequencing can alter the workflow, cost, analysis, and interpretation of genomics associated with PCC/PGL.

* Corresponding author at: Head of Pathology, Griffith Medical School, Gold Coast Campus, Gold Coast QLD 4222, Australia.
E-mail address: a.lam@griffith.edu.au (A.K.-Y. Lam).

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1. Introduction

Gene sequencing plays important roles in identify pathological mutations which are important to the understanding of the pathogenesis of human diseases as well as planning targeted gene therapies for patients with cancer. A neuroendocrine tumour that arises in the chromaffin cells in adrenal medulla is termed phaeochromocytoma (PCC). Extra-adrenal tumours arising from chromaffin cells are paragangliomas (PGL) (Lam, 2015). Currently, twenty-nine genes are known to be associated with PCC/PGL and the list is growing (Pillai et al., 2016a,b). 40% of PCC/PGL is associated with germline mutation in one of the susceptibility genes and the remaining 60% are thought to be sporadic cases (Favier et al., 2015). Overall, known genetic mutations account for the pathogenesis of approximately 60% of PCC/PGLs (Lam, 2015). These genetic changes of PCC/PGL are one of the revisions incorporated in the current World Health Classification of endocrine tumours (Lam, 2017). Thus, the patients with this group of tumours will benefit from studying the mutations in the tumours by gene sequencing.

The American Society of Clinical Oncology suggests that all patients with PCC and PGLs should be submitted to genetic screening as these tumours present with high degree of genetic heterogeneity and heritability (Burnichon et al., 2016). The aim of these clinical genetic screening is to identify patients who are carriers of disease-causing mutations or to test tumour tissue for the presence of genetic alterations in PCC/PGL which may be targeted for therapeutic approaches (Toledo and Dahia (2015)). With the increase in number of susceptibility genes identified in PCC/PGL, single gene testing method was not practical in clinical settings. The use of high-throughput technology or next generation sequencing (NGS), where simultaneously multiple genes could be tested for mutation making it easier in detecting predisposing genes of PCC/PGL (Toledo and Dahia (2015)). Table 1 summarizes the PCC/PGL genes discovered through NGS. Thus, the use of NGS has vital implications in unveiling the genetic mystery associated with the molecular pathogenesis of PCC/PGLs.

In this review, we will summarize three generations of sequencing, different platforms of next generation sequencing (NGS) as well as the applications, limitations and future perspectives of next generation sequencing with regards to PCC/PGL.

2. First generation sequencing and limitations

DNA sequencing is generally referred to the sequencing of nucleotides within a DNA molecule using laboratory methods (Sanger et al., 1977). In 1977, two methods of DNA sequencing were described; the first one was described by Maxam and Gilbert using the chemical breakage, radioisotope labeling and gel electrophoresis method to sequence DNA (Maxam and Gilbert, 1977). The second one was described by Sanger who utilised the dideoxynucleotide analogues as specific chain-terminating inhibitors of DNA polymerase for sequencing method (Sanger et al., 1977).

Sanger's method, when compared to Maxam and Gilbert method, was the most preferred among scientists and researchers due to its high efficiency and involvement of non-toxic chemicals. It is being labelled as the first-generation sequencing (FGS) technology (Mardis, 2014). Also, Sanger is considered the father of sequencing technologies as Sanger's sequencing methods and technologies have been later improved to build future sequencing platforms.

Sanger sequencing had limited applications because of some technical limitations in its workflow in terms of throughput (Rizzo and Buck, 2012). Throughput is a function of sequencing reaction time, the number of sequencing reactions that can be run in parallel, and lengths of sequences read by each reaction (Rizzo and Buck, 2012). In Sanger sequencing, the throughput is approximately 115 kb/day which means the instrument can only read 96 reactions in parallel (Rizzo and Buck, 2012). Also, the cost of sequencing of an entire human genome by Sanger is approximately $5 to 30 million USD. The time taken to accomplish the task is estimated to be around 60 years if using a single instrument (Bennett et al., 2005).

3. Birth of second generation sequencing/next generation sequencing (SGS/NGS)

Following the successes of human genome project in 2005, massive parallel sequencing systems called the next-generation sequencing (NGS) was developed to reduce the cost and time of genome sequencing.

SGS/NGS refers to the high-throughput DNA sequencing technologies which are capable of sequencing large numbers of different DNA sequences in a single/parallel reaction (Mardis, 2013). NGS technologies such as 454 Life Sciences, Illumina and Applied Biosciences are the three principal systems, which are commercially available (Tables 2 and 3). Their specific features, as well as their sequencing chemistries (Fig. 1) are briefly described below.

3.1. 454 Roche/Life sciences

The 454Roche/Life Sciences was the first commercially introduced NGS system available in 2005. It is the first non-Sanger technology to sequence human genome (Rothberg and Leamon, 2008). This system utilises the mechanism of pyrosequencing and it depends on the detection of pyrophosphate released during nucleotide incorporation (Margulies et al., 2005). In this method, template DNA molecules are generated by fragmentation or polymerase chain reaction (PCR) (Margulies et al., 2005; Moorthie et al., 2011). Universal adaptors are ligated to the fragmented end if fragmentation method is used or adaptors can be built into the primers if PCR is used (Rothberg and Leamon, 2008). The prepared fragments are then hybridised on to special beads. Then, a mixture of these beads, PCR reagents and oil is agitated to form tiny oil reaction chambers. This is then subjected to thermal cycling in order to amplify the DNA template present on the surface of each bead (Ambardar et al., 2016).

Pico Titre Plate is a specially designed plate used in the 454 Life Sciences platform to carry out the sequencing reaction. It comprises of millions of microscopic wells, and the beads are loaded onto these wells along with all the reagents required for sequencing except the nucleotides (Moorthie et al., 2011). The final sequencing step in this system then proceeds with the chronological addition of each individual nucleotide in an order of A, C, G and T (Harrington et al., 2013). However, if a nucleotide is added by DNA polymerase into the growing DNA strand, an inorganic phosphate ion is released resulting in the discharge of a flash of light hence the name pyrosequencing and is detected by a camera across the whole plate (Ballester et al., 2016). This whole process is repeated several hundred times to build the temporal image sequence which is then computationally converted into sequence reads (Liu et al., 2012).

At the beginning, the read length of Roche 454 system is usually between 100 to 150 base pairs with 200,000 reads, and output of 20 Mb per run. Later, its read length got increased to 700 base pairs with an output of 0.7 G data per run with the introduction of 454 GS FLX Titanium system in 2008 (Liu et al., 2012).

The major advantage of this system is its speed as it takes only 10 h from sequencing start till completion. However, this system has a relatively high error rate in terms of poly-bases longer than 6 bp and the high cost of the reagents still remains as a challenge for using this system in clinical use (Liu et al., 2012; Ballester et al.,