MOLECULAR ONCOLOGY XXX (2015) 1–9

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Review

Non-invasive detection of genome-wide somatic copy number alterations by liquid biopsies

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ARTICLE INFO

Article history:
Received 16 October 2015
Received in revised form
26 November 2015
Accepted 3 December 2015
Available online ■

Keywords:
Circulating tumor cells (CTCs)
Circulating tumor DNA (ctDNA)
Plasma DNA
Whole-genome sequencing
Somatic copy number alterations

ABSTRACT

Liquid biopsies, i.e. the analysis of circulating tumor cells (CTCs) or circulating tumor DNA (ctDNA), are evolving into promising clinical tools. Indeed, a plethora of liquid biopsy technologies to deduce non-invasively characteristics of the tumor genome from the peripheral blood have been developed over the last few years. For example, liquid biopsies have been used to assess the tumor burden, to monitor the evolution of tumor genomes, to unravel mechanisms of resistance, to establish the tumor heterogeneity, and for the identification of prognostic and predictive markers. In this review we focus on methods to establish genome-wide profiles of somatic copy number alterations (SCNAs) from plasma DNA and show how they provide novel insights into the biology of cancer and their impact on the management of patients.

1. Introduction

Cancer cells harbor a plethora of somatic alterations in their tumor genomes, such as base substitutions, insertions or deletions of small DNA segments (indels), copy number changes of large chromosomal regions, structural rearrangements, such as translocations, and epigenetic changes which alter chromatin structure and gene expression (Stratton et al., 2009; Vogelstein et al., 2013). Furthermore, cancer genomes are often unstable and accumulate new changes depending on exerted selection pressures. Therefore, tools allowing the monitoring of tumor genomes with easy means should be of great importance.

To this end, considerable progress has recently been achieved with the analyses of circulating tumor cells (CTCs) and plasma DNA. The cell-free fraction of blood consists in patients with cancer of circulating tumor DNA (ctDNA) and DNA fragments released from normal cells in variable proportions (Crowley et al., 2013; Diaz and Bardelli, 2014; Heitzer et al., 2013c, 2015; Schwarzenbach et al., 2011). The variable allele fraction of mutant DNA fragments may reflect tumor dynamics (Diehl et al., 2005) and has an impact on the selection of methods for their subsequent analyses (Belic et al., 2015; Heitzer et al., 2015).

A specific biomarker of disease burden are mutations which have previously been identified in the primary tumor,

This is a contribution to the special issue edited by Klaus Pantel and Catherine Alix-Panabieres, Liquid Biopsies.

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http://dx.doi.org/10.1016/j.molonc.2015.12.004
1574-7891/

and several studies have focused on the detection of such specific and predetermined mutations in corresponding peripheral blood from the same patient (Diehl et al., 2005, 2008a, 2008b; Leary et al., 2010; McBride et al., 2010; Nawroz et al., 1996; Yung et al., 2009). Tumor-specific structural chromosomal rearrangements, i.e. breakpoints, identified by whole-genome sequencing of primary tumors, which are then subsequently used to monitor in plasma of the respective patients minimal residual disease (MRD) by high-sensitive PCR approaches have a particularly high specificity (Leary et al., 2010; McBride et al., 2010; Olsson et al., 2015). Various approaches for the non-invasive identification of somatic mutations in blood at high resolution have been developed (Bettegowda et al., 2014; Dawson et al., 2013; Forshew et al., 2012; Misale et al., 2012; Murtaza et al., 2013; Newman et al., 2014; Thierry et al., 2014). Mutation analyses assisted in the elucidation of resistance mechanisms (Diaz et al., 2012; Misale et al., 2014, 2012; Murtaza et al., 2013; Siravegna et al., 2015), for the assessment of tumor heterogeneity (Reinert et al., 2015), and establishment of methylation patterns (Chan et al., 2013a; Sun et al., 2015). Furthermore, we and others explored approaches for analyses of somatic copy number alterations (SCNAs) at a genome-wide scale (Chan et al., 2013b; Heitzer et al., 2013b; Heitzer et al., 2013d; Leary et al., 2012; Murtaza et al., 2013).

Indeed, among the outstanding characteristics of cancer genomes are their frequent SCNAs and often extensive aneuploidies (Stratton et al., 2009; Vogelstein et al., 2013). Their identification and characterization are of utmost importance for basic research, understanding disease mechanisms, and tumor classification. Furthermore, they may contribute to the identification of prognostic and predictive tumor markers, which is of significance for personalized medicine, or "biologically personalized therapeutics" (Cherny et al., 2014). Here, we review methods and strategies for copy number detection and interpretation as well as their advantages and limitations.

1.1. *Relevance and biology of somatic copy number alterations in cancer*

90% of solid tumors and 50% of blood-related cancers are aneuploid and have SCNAs (Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer; http://cgap.nci.nih.gov/Chromosomes/Mitelman; (Beroukhim et al., 2010)). SCNAs alter a larger percentage of the genome than any other somatic genetic alterations (Beroukhim et al., 2010; Stratton et al., 2009; Vogelstein et al., 2013). SCNAs comprise losses (i.e. deletions), gains (e.g. duplications), and high-level amplifications (Figure 1). In principle, gains and losses are copy number changes of any length and amplitude. However, a "gain" is usually a relatively moderate copy number increase (e.g. trisomy or tetrasomy), whereas an "amplification" is a high level gain with sometimes up to several hundred copies (Stratton et al., 2009), frequently occurring of only a restricted size. There is no exact definition for an upper size limit of a focal amplification and previous studies have analyzed focal SCNAs with a size of up to 85 Mb (Beroukhim et al., 2010) or shorter than the chromosome arm (Zack et al., 2013). Similarly, homozygous deletions are usually also only observed for relatively small regions and accordingly a correlation between amplitude and size for both high-level amplifications and homozygous deletions has been reported (Beroukhim et al., 2010).

SCNAs may affect activation of oncogenes (OGs) or inactivation of tumor suppressor genes (TSGs) (Beroukhim et al., 2010; Stratton et al., 2009; Vogelstein et al., 2013). The loss of TSGs and gain of OGs may propel further karyotype changes, including whole or partial chromosome gains and losses. This may result in clonal aneuploidy karyotype patterns, which are frequently characteristic of a specific cancer (Davoli et al., 2013; Santaguida and Amon, 2015).

When applying novel parameters for predicting TSGs and OGs, TSGs were found to be enriched in recurring focal deletions, whereas OGs were enriched in amplifications and depleted from focal deletions. Hence, recurrent focal deletions and amplifications both may represent "Cancer Gene Islands", which are characterized by particular densities of TSGs and OGs (Solimini et al., 2012) (Figure 2a).

However, gaining or losing large chromosomal regions or even whole chromosomes affects not specifically TSGs and OGs, but results frequently yet not universally in expression changes of large numbers of genes. Indeed, recent large-scale integrated analyses of copy number and gene expression have found that SCNAs comprise a major mechanism driving carcinogenesis in epithelial cancers, such as breast and prostate carcinoma (Curtis et al., 2012; Grasso et al., 2012; Taylor et al., 2010). A study of 2000 primary breast tumors has reported that the expression landscape was dominated by *cis*- and *trans*-acting acquired SCNAs and the patterns of salient SCNAs even allowed the definition of new breast-cancer subtypes (Curtis et al., 2012). In prostate cancer genome-wide comprehensive analyses have revealed that outlying expression coincided with copy number events (Grasso et al., 2012; Taylor et al., 2010). Hence, the phenotypes of aneuploidy cells are caused by gene imbalances and by simultaneous changes in the gene dosage of many genes (Santaguida and Amon, 2015).

Indeed, the distribution and potency of TSGs and OGs on chromosomes was shown to influence the frequency of whole-chromosome arm SCNAs in cancer. Applying a newly developed score that measures positive and negative growth and survival potential that wild-type OGs or TSGs normally impart to the respective chromosome arm it was calculated how SCNAs might impact cancer evolution by altering this balance (Davoli et al., 2013).

Importantly, Davoli et al., (2013) suggested the existence of two classes for both TSGs and OGs. One TSG class is haploinsufficient (Haploinsufficiency: loss of one copy of a gene causes in a diploid organism a phenotype) and contributes to sporadic cancer, whereas the other class is haplosufficient without significant contribution to cancer. In case of the first class TSGs cancer may occur by loss of one functional allele, which may produce a selectable phenotype, whereas the latter class refers to the familial TSGs and the two-hit model of tumorigenesis (Knudson, 2001). Estimates suggest ~30% haploinsufficiency overall among human genes (Davoli et al., 2013). In fact, the vast majority, if not all, of sporadic TSGs are likely to be haploinsufficient, and these genes cannot be identified by mutation analyses but rather by systematic mapping of recurrently deleted chromosomal regions (Davoli et al.,

Please cite this article in press as: Heitzer, E., et al., Non-invasive detection of genome-wide somatic copy number alterations by liquid biopsies, Molecular Oncology (2015), http://dx.doi.org/10.1016/j.molonc.2015.12.004