MOLECULAR ONCOLOGY 6 (2012) 347–359

available at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/molonc

Structural and genic characterization of stable genomic regions in breast cancer: Relevance to chemotherapy

Nicole I. Park[a], Peter K. Rogan[b,c], Heather E. Tarnowski[a], Joan H.M. Knoll[a,d,*]

[a]Department of Pathology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada
[b]Department of Biochemistry, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada
[c]Department of Computer Science, University of Western Ontario, London, ON, Canada
[d]Molecular Pathology, Laboratory Medicine Program, London Health Sciences Centre, ON, Canada

A R T I C L E I N F O

Article history:
Received 5 August 2011
Received in revised form
28 November 2011
Accepted 2 January 2012
Available online 15 January 2012

Keywords:
Breast cancer
Genomic stability
Chemotherapy
Genetic selection
Array comparative genomic hybridization
Gene expression
Bioinformatics
Fluorescence in situ hybridization

A B S T R A C T

Background: Cancer genomes accumulate frequent and diverse chromosomal abnormalities as well as gene mutations but must maintain the ability to survive *in vivo*. We hypothesize that genetic selection acts to maintain tumour survival by preserving copy number of specific genes and genomic regions. Genomic regions and genes that remain unaltered in copy number and expression, respectively, may be essential for maintaining tumour survival.

Methods: We analyzed copy number data of 243 previously reported breast tumours and computationally derived stable copy number regions. To identify genes in stable copy number regions with nominal changes in expression, datasets for tumour and normal samples were compared. Results were replicated by analysis of a series of independent copy number, expression and genomic sequencing studies. A subset of stable regions, including stable paralogous regions, were confirmed by quantitative PCR and fluorescence in situ hybridization (FISH) in 5 breast cancer cell lines. We deduced a comprehensive set of dually stable genes (i.e. maintaining nominal copy number and expression) which were categorized according to pathway and ontology assignments. The stability of genes encoding therapeutic drug targets was also assessed.

Results and Conclusion: Tumour genome analysis revealed 766 unstable (amplified and/or deleted) and 812 stable contiguous genomic regions. Replication analysis of an independent set of 171 breast tumours confirmed copy number stability of 1.3 Gb of the genome. We found that 5804 of these genes were dually stable. The composition of this gene set remained essentially unchanged (<2% reduction) after accounting for commonly mutated breast cancer genes found by sequencing and differential expression. The stable breast cancer genome is enriched for cellular metabolism, regulation of gene expression, DNA packaging (chromatin and nucleosome assembly), and regulation of apoptosis functions. Stable genes participating in multiple essential pathways were consistently found to be targets of chemotherapies. Preservation of stable, essential genes may be related to the effectiveness of certain chemotherapeutic agents that act on multiple gene products in this set.

© 2012 Federation of European Biochemical Societies.
Published by Elsevier B.V. All rights reserved.

* *Corresponding author*. Department of Pathology, Schulich School of Medicine and Dentistry, University of Western Ontario, Dental Sciences Building, Rm 4044, 1151 Richmond Street, London, ON, N6A 5C1 Canada. Tel.: +1 519 661 2111x86407.
E-mail address: jknoll3@uwo.ca (J.H.M. Knoll).

1574-7891/$ – see front matter © 2012 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.molonc.2012.01.001

1. Introduction

Mutation studies establish which essential gene products are critical for growth, and development of tumours. Despite extensive genomic instability, presumably, a minimal set of gene products are required for tumour cell survival. Loss-of-function mutations required for the proliferation and survival of cancer cells have been investigated using RNA interference (Ngo et al., 2006; Silva et al., 2008). Functional genetic analyses have identified causal cancer genes and much effort has been made to determine their contribution to the tumourigenic phenotype.

Human cancers arise from the accumulation of numerous genetic and epigenetic alterations, which lead to dysregulation of protein-coding genes and interacting genes within a pathway (Schafer et al., 2009). Microarray studies assess abnormalities in copy number of specific genes (Hicks et al., 2006), expression (Perou et al., 2000), and methylation status (Feinberg and Tycko, 2004; Widschwendter and Jones, 2002). Genomic rearrangements, deletions, amplifications, and point mutations of genes regulating cell growth, apoptosis and DNA repair are responsible for unregulated proliferation (Vogelstein and Kinzler, 2004). As well, alterations in oncogenes and tumour-suppressor genes contribute to tumourigenesis (Davies et al., 2002; Friedberg, 2003; Nowell, 2002; Santarosa and Ashworth, 2004). Common targets for amplification and deletion include *ERBB2*, *MYC*, *CDKN2A*, *PTEN*, and *SMAD4* (Collins and Groudine, 1982; Hahn et al., 1996; Kamb et al., 1994; Li et al., 1997; Slamon et al., 1987; Steck et al., 1997). In breast tumours, genomic regions that are consistently abnormal have been termed "saw-tooth" and "firestorm regions" because they possess the highest frequencies of gains and losses of genomic sequences (Hicks et al., 2006). However, investigation of genes with little or no variation in copy number or expression has not been a focus of cancer studies, even though they may also contribute to maintenance of the tumour phenotype.

Confronted with frequent chromosome instability and gene mutation, some tumour cell lineages are surprisingly resilient to autophagy and apoptosis. We investigate the composition of the stable gene set in breast tumours which presumably contributes to their survival, regardless of whether they are derived from cancer stem cells or from source cells that have avoided inactivation of essential genes. We have characterized regions of breast cancer genomes that share stable copy number (Chin et al., 2007; Hicks et al., 2006) and exhibit levels of expression similar to matched normal tissues (Turashvili et al., 2007; Naderi et al., 2007). We address whether these stable regions encode essential gene products by determining if standard breast cancer chemotherapies kill cancer cells by depriving tumours of these functions.

2. Materials and methods

2.1. *Definition of stable and unstable genomic regions in the breast cancer genome*

Copy number and expression were analyzed from independent array comparative genomic hybridization datasets (aCGH): by a Representational Oligonucleotide Microarray Analysis (ROMA; GEO GPL7313) and a custom 30K 60-mer oligonucleotide array (GEO GPL5737). The ROMA platform contained approximately 85,000 probes with an approximately uniform genomic distribution (Lisitsyn et al., 1993; Lucito et al., 2003). The data consist of 2847 probes that detected autosomal deletions and amplifications in 243 primary breast carcinoma tissues (Hicks et al., 2006). The custom array contained 60-mer oligonucleotides representing 28,830 unique genes (van den Ijssel et al., 2005). In this aCGH platform, 1684 highly recurrent altered regions were found in 171 primary breast tumours (Chin et al., 2007).

Autosomal variations in copy number among multiple tumours were determined relative to a normal diploid male DNA (Hicks et al., 2006) or to a reference pool of 50 randomized tumours (Chin et al., 2007). In both studies, at least 10% of the tumours were required to display a consistent increase or decrease of at least one copy of the target locus. Neither study (Chin et al., 2007; Hicks et al., 2006) controlled for tumour subtype or heterogeneity. The requirement for ubiquitous genomic stability across all breast tumour subtypes is expected to identify common genomic intervals that are essentially unaltered in the most prevalent types of tumours (however, conclusions about stability in individual subtypes may not be valid).

ROMA probe IDs were ordered by genomic coordinate and hybridization copy number (based on NCBI Build 36/hg18 assembly). Adjacent probes with identical copy numbers (either increased or decreased) were grouped to form contiguous intervals with the same unstable genotypes. This same approach was taken to cluster intervals of gains or losses less than 105 kb apart using an independent dataset (Chin et al., 2007).

Stable genomic intervals were inferred by complementing genomic coordinates of clusters of tightly linked unstable intervals. Genes located within stable and unstable regions were determined by convolving the genomic coordinates of all known protein-coding genes (CCDS; build Hs36.3) with those of stable and unstable regions using the Galaxy metaserver (http://main.g2.bx.psu.edu). The history and results of the operations used to derive this and other genomic datasets are available on our laboratory website (http://cancer.cytognomix.org/stable). Genes that overlapped the interface between adjacent stable and unstable intervals were classified as unstable. Although conservative, this approach avoided false assignments of unstable regions as stable. Stable chromosomal regions were further characterized by comparing the cumulative stability across each chromosome to the frequency of recurrent cytogenetic abnormalities in breast cancer (Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer; http://cgap.nci.nih.gov/Chromosomes/Mitelman; $n = 5328$, Sept 2010 version).

2.2. *Gene expression analysis with copy number data across breast cancer subtypes*

To identify genes in stable regions with nominal expression, datasets for tumour and normal samples were compared. These consisted of either matched tumour and normal

Download English Version:

https://daneshyari.com/en/article/2145798

Download Persian Version:

https://daneshyari.com/article/2145798

Daneshyari.com