MOLECULAR ONCOLOGY 9 (2015) 1186–1193

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/molonc

Telomere fusion threshold identifies a poor prognostic subset of breast cancer patients

*K. Simpson, R.E. Jones, J.W. Grimstead, R. Hills, C. Pepper, D.M. Baird**

Institute of Cancer & Genetics, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK

ARTICLE INFO

Article history:
Received 16 July 2014
Received in revised form
29 January 2015
Accepted 6 February 2015
Available online 25 February 2015

Keywords:
Breast cancer
Prognosis
Genome instability
Telomere

ABSTRACT

Telomere dysfunction and fusion can drive genomic instability and clonal evolution in human tumours, including breast cancer. Telomere length is a critical determinant of telomere function and has been evaluated as a prognostic marker in several tumour types, but it has yet to be used in the clinical setting. Here we show that high-resolution telomere length analysis, together with a specific telomere fusion threshold, is highly prognostic for overall survival in a cohort of patients diagnosed with invasive ductal carcinoma of the breast ($n = 120$). The telomere fusion threshold defined a small subset of patients with an extremely poor clinical outcome, with a median survival of less than 12 months (HR = 21.4 (7.9–57.6), $P < 0.0001$). Furthermore, this telomere length threshold was independent of ER, PGR, HER2 status, NPI, or grade and was the dominant variable in multivariate analysis. We conclude that the fusogenic telomere length threshold provides a powerful, independent prognostic marker with clinical utility in breast cancer. Larger prospective studies are now required to determine the optimal way to incorporate high-resolution telomere length analysis into multivariate prognostic algorithms for patients diagnosed with breast cancer.

1. Introduction

The clinical management of breast cancer is informed by prognostic and predictive markers, which allow breast tumours to be classified into subtypes that can display distinct clinical outcomes. These markers include histopathological criteria such as tumour size, grade and lymph node metastasis: as well as the molecular markers, such as oestrogen receptor (ER) and progesterone receptor (PGR), that predict response to treatment with endocrine therapy, or human epidermal growth factor receptor 2 (HER2) that predicts response to HER2 antagonists (Subramaniam and Isaacs, 2005). These data are used in combination with multivariate algorithms to inform on treatment options and to provide prognostic information (Mook et al., 2009). These markers can be augmented by gene expression profiling that has allowed the identification of additional molecular subtypes, which can inform about treatment options in specific clinical subsets (Hennessy et al., 2009). Whilst these markers can inform the clinical management of breast cancer, they are unable to provide definitive individualised prognostic information.

Whole genome sequence analysis of breast tumours has revealed further levels of genetic heterogeneity that can impact on clinical outcome, it is apparent from these studies that increasing genomic complexity confers a poorer prognosis (Curtis et al., 2012). The underlying mechanisms driving

* *Corresponding author*. Tel.: +44 (0) 29 20 687 038.
E-mail address: bairddm@cardiff.ac.uk (D.M. Baird).
http://dx.doi.org/10.1016/j.molonc.2015.02.003

genomic instability and selection for complex genomes in breast cancer is not entirely clear, but likely includes DNA repair and checkpoint defects, as well as telomere dysfunction (Kwei et al., 2010). Telomeres are structures that cap the ends of chromosomes and prevent aberrant repair of the natural chromosome end by the DNA double strand break repair apparatus (de Lange, 2005). Telomere erosion, as a function of cell division, can lead to the formation of short dysfunctional telomeres that have lost the end-capping function and are capable of fusion with other telomeres or non-telomeric loci (Capper et al., 2007; Counter et al., 1992). The presence of short dysfunctional telomeres, which are capable of fusion and associated with genomic complexity, have been detected in many tumour types, including breast cancer (Chin et al., 2004; Lin et al., 2010; Roger et al., 2013). These data are consistent with tumour cells undergoing a period of 'telomere crisis' during the progression to malignancy, which drives large-genomic rearrangements and creates the diversity on which clonal selection can operate. Telomere dysfunction and fusion may represent a common mechanism of genomic instability facilitating progression in a broad range of tumour types.

Importantly, short dysfunctional telomeres have been detected in early-stage tumours (Lin et al., 2010; Meeker et al., 2004b), as well as in premalignant lesions. This indicates that telomere dysfunction can precede disease progression and is not simply a biomarker of advanced disease. Indeed, recent evidence suggests that short telomeres may be an inherent property of a proportion of cells in which the tumour initiating event occurred (Roger et al., 2013). Telomere length in early stage lesions varies considerably between different tumour clones, leading to the hypothesis that clones harbouring short dysfunctional telomeres exhibit a mutator phenotype that can drive clonal evolution and progression: whereas those exhibiting longer telomeres, may have a more stable genome and be less prone to clonal evolution (Roger et al., 2013). Thus, by informing on the propensity of a tumour to undergo clonal evolution, the telomere length of early-stage tumours, has the potential to provide prognostic information. We have recently examined this hypothesis in chronic lymphocytic leukaemia (CLL), in which we had previously identified that telomere shortening and dysfunction can occur prior to clinical progression (Lin et al., 2010). We established the telomere length threshold below which telomere fusion could be detected and we used this to stratify patients based on telomere length (Lin et al., 2014). These data show that telomere lengths below the fusion threshold are highly prognostic and that the mean of the fusogenic range (2.26 kb) provided the optimum prognostic resolution. Indeed, telomere length below the fusion threshold was the most powerful predictor of survival in CLL and this was particularly prognostic in early-stage patients (HR = 19.3 (17.8–802.5), $P < 0.0001$) (Lin et al., 2014). Given the prognostic significance of our findings in CLL and that telomere dysfunction has been implicated in many tumour types, we wanted to establish whether our threshold for telomere dysfunction could predict outcome in other common solid human cancers. Here we show that the telomere fusion threshold, established in CLL, identifies a subgroup of breast cancer patients with an extremely poor clinical outcome.

2. Materials and methods

2.1. Breast cancer cohort

DNA samples extracted from frozen tissue blocks obtained from surgically resected Invasive Ductal carcinoma of the breast were obtained with ethical approval from the Wales Cancer Bank. A certified histopathologist determined percentage tumour in each section as detailed in Supplementary Table 1. DNA extraction was performed using the Qiagen TissueLyser to homogenize the tissue and the QIAamp DNA mini kit (Qiagen, Cat#: 51306) according to the manufacturers guidelines. The median follow-up of the cohort was 4.6 years and the patients' clinical/laboratory characteristics are summarized in Table 1 and provided in full in Supplementary table 1.

2.2. Telomere length analysis

For telomere length analysis at the XpYp telomere we used the single telomere length analysis (STELA) assay as previously described (Baird et al., 2003; Capper et al., 2007); for three samples that displayed bimodal telomere length distributions, we used the mean of lower modal distribution.

Table 1 – Clinical characteristics of the invasive ductal breast carcinoma cohort (n = 120).

Factor	Subset	Number
Median age		60.5 years
Range		33–87 years
Median follow-up		4.6 years
Grade	I	11
	II	47
	III	62
ER status	negative	30
	positive	89
	not determined	1
PGR status	negative	36
	positive	41
	not determined	43
NPI status	<3.4	14
	>3.4/<5.4	37
	>5.4	18
	not determined	51
HER2 status	negative	78
	positive	27
	not determined	15
Adjuvant chemotherapy	negative	39
	positive	81
Adjuvant radiotherapy	negative	31
	positive	89
Adjuvant hormone therapy	negative	26
	positive	91
	unknown	3

ER = estrogen receptor.
PGR = progesterone receptor.
NPI = Nottingham Prognostic Index.
HER2 = human epidermal growth factor receptor 2.