Cancer Research

Tumor and Stem Cell Biology

Hypoxia Drives Breast Tumor Malignancy through a TET–TNFα–p38–MAPK Signaling Axis

Min-Zu Wu, Su-Feng Chen, Shin Nieh, Christopher Benner, Luo-Ping Ger, Chia-Ing Jan, Li Ma, Chien-Hung Chen, Tomoaki Hishida, Hong-Tai Chang, Yaoh-Shiang Lin, Nuria Montserrat, Pedro Gascon, Ignacio Sancho-Martinez, and Juan Carlos Izpisua Belmonte

DOI: 10.1158/0008-5472.CAN-14-3208 Published 15 September 2015

Abstract

Hypoxia is a hallmark of solid tumors that drives malignant progression by altering epigenetic controls. In breast tumors, aberrant DNA methylation is a prevalent epigenetic feature associated with increased risk of metastasis and poor prognosis. However, the mechanism by which hypoxia alters DNA methylation or other epigenetic controls that promote breast malignancy remains poorly understood. We discovered that hypoxia deregulates TET1 and TET3, the enzymes that catalyze conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), thereby leading to breast tumor–initiating cell (BTIC) properties. TET1/3 and 5hmC levels were closely associated with tumor hypoxia, tumor malignancy, and poor prognosis in breast cancer patients. Mechanistic investigations showed that hypoxia leads to genome-wide changes in DNA hydroxymethylation associated with upregulation of TNFα expression and activation of its downstream p38–MAPK effector pathway. Coordinate functions of TET1 and TET3 were also required to activate TNFα–p38–MAPK signaling as a response to hypoxia. Our results reveal how signal transduction through the TET–TNFα–p38–MAPK signaling axis is required for the acquisition of BTIC characteristics and tumorigenicity *in vitro* and *in vivo*, with potential implications for how to eradicate BTIC as a therapeutic strategy. *Cancer Res; 75(18); 3912–24. ©2015 AACR.*

Introduction

Breast cancer is the most common malignancy among women and exhibits a high rate of heterogeneity (1). Although mortality of patients with breast cancer in recent years has significantly decreased, tumor malignancy of breast cancer still leads to a poor prognosis and limits therapeutic options. It was proposed that tumor-initiating cells (TIC), defined by their abilities for tumor-forming and self-renewing, account for tumor malignancy (2). Recently, the population of TICs has been identified in numerous tumors, including those of brain, head and neck, and lung cancer, through various molecular markers, side population, and enzyme activity (3–5). Likewise, breast tumor–initiating cells (BTIC) have been isolated by sorting for $CD44^{High}/CD24^{Low}$ cells, high aldehyde dehydrogenase1 (ALDH1) activity, or enriched in anchorage-independent conditions (6–8). They were found to share a high degree of similarity of characteristics with stem cells as well as tumorigenic capacity (9). High content of BTICs are enriched with high grade of breast tumor and associated with enhanced invasiveness, chemoresistance, and metastasis (10), supporting the concept that BTICs account for tumor malignancy of breast cancer (11).

Hypoxia, a microenvironment stress existing in various solid tumors, has been recognized as an important factor promoting tumor malignancy (12). Recent reports demonstrating the association of hypoxia with high-grade breast tumor and poor prognosis of patients with breast cancer have suggested an important role for hypoxia in breast cancer (13). Noticeably, hypoxia has been demonstrated to be associated with a stem-like phenotype in breast cancer and provides a breeding ground for BTICs (14). This is consistent with the fact that TICs are supported by their niche where they are regulated by complex interactions with multiple factors derived from the tumor microenvironment (15).

One key mechanism by which hypoxia regulates tumor malignancy is alteration of the cancer epigenome, which provides selective advantages for cancer cells during tumorigenesis (16, 17). Aberrations in DNA methylation and proteins involved in controlling DNA methylation are associated with tumor malignancy and prognosis of patients (18). However, the mechanism by which hypoxia regulates DNA methylation and key enzymes in regulating tumor malignancy remains largely unknown. The Ten-Eleven Translocation (TET) family of enzymes convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) for the demethylation of mammalian DNA (19). The TET family comprises three members: TET1, TET2, and TET3. The *TET1* gene was firstly identified as a fusion partner of MLL in acute myeloid leukemia associated with a chromosome translocation. The expression of TET1 is required for ESC pluripotency and normal differentiation during ESC lineage specification (20). TET2 plays an important role in hematopoiesis. Loss-of-function of TET2 has been reported as one of the most frequent genetic defects in myeloid malignancies (21). In addition, TET3, but not TET1 or TET2, highly expressed in oocytes and zygotes, is essential for epigenetic reprogramming of the zygotic paternal DNA (22). Despite these findings suggesting critical roles that TET family proteins play during biologic processes, it is unclear whether TET proteins act as key factors in regulating hypoxia-enhanced tumor malignancy as well as tumor-initiating capabilities.

In this study, we reveal an epigenetic mechanism centered on hypoxia-induced activation of the TNFα–p38–MAPK signaling axis leading to breast tumor malignancy, and highlight that TET1/3 and 5hmC might serve as prognostic biomarkers for breast cancer. The inhibitory effect of blocking the TET–TNFα–p38–MAPK signaling pathway on BTIC characteristics and tumorigenicity indicates a potential strategy to improve targeted therapy in breast cancer.