Free Radical Biology and Medicine 80 (2015) 12–26

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Reactive oxygen species-mediated breast cell carcinogenesis enhanced by multiple carcinogens and intervened by dietary ergosterol and mimosine

Lenora Ann Pluchino [a,b], Amethyst Kar-Yin Liu [a], Hwa-Chain Robert Wang [a,b,*]

[a] Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, 2407 River Drive, Knoxville, TN 37996, USA
[b] Graduate School of Genome Science and Technology, University of Tennessee, Knoxville, TN 37996, USA

ARTICLE INFO

Article history:
Received 27 June 2014
Received in revised form
11 December 2014
Accepted 12 December 2014

Available online 20 December 2014Keywords
Breast cell carcinogenesis
Carcinogens
Dietary prevention
Antioxidants

ABSTRACT

Most breast cancers occur sporadically due to long-term exposure to low-dose carcinogens in the diet and the environment. Specifically, smoke, polluted air, and high-temperature cooked meats comprise multiple carcinogens, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), benzo[α]pyrene (B[α]P), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). We sought to determine if these carcinogens act together to induce breast cell carcinogenesis, and if so, whether noncytotoxic dietary agents could intervene. We demonstrated that coexposure to physiologically achievable doses of NNK, B[α]P, and PhIP (NBP) holistically enhanced initiation and progression of breast cell carcinogenesis. Reactive oxygen species (ROS) and activation of the ERK pathway were transiently induced by NBP in each exposure, and cross talk between reinforced ROS elevation and ERK activation played an essential role in increased DNA oxidation and damage. After cumulative exposures to NBP, this cross talk contributed to enhanced initiation of cellular carcinogenesis and led to enhanced acquisition of cancer-associated properties. Using NBP-induced transient changes, such as ROS elevation and ERK pathway activation, and cancer-associated properties as targeted endpoints, we revealed, for the first time, that two less-studied dietary compounds, ergosterol and mimosine, at physiologically achievable noncytotoxic levels, were highly effective in intervention of NBP-induced cellular carcinogenesis. Combined ergosterol and mimosine were more effective than individual agents in blocking NBP-induced transient endpoints, including ROS-mediated DNA oxidation, which accounted for their preventive ability to suppress progression of NBP-induced cellular carcinogenesis. Thus, dietary components, such as mushrooms containing ergosterol and legumes containing mimosine, should be considered for affordable prevention of sporadic breast cancer associated with long-term exposure to environmental and dietary carcinogens.

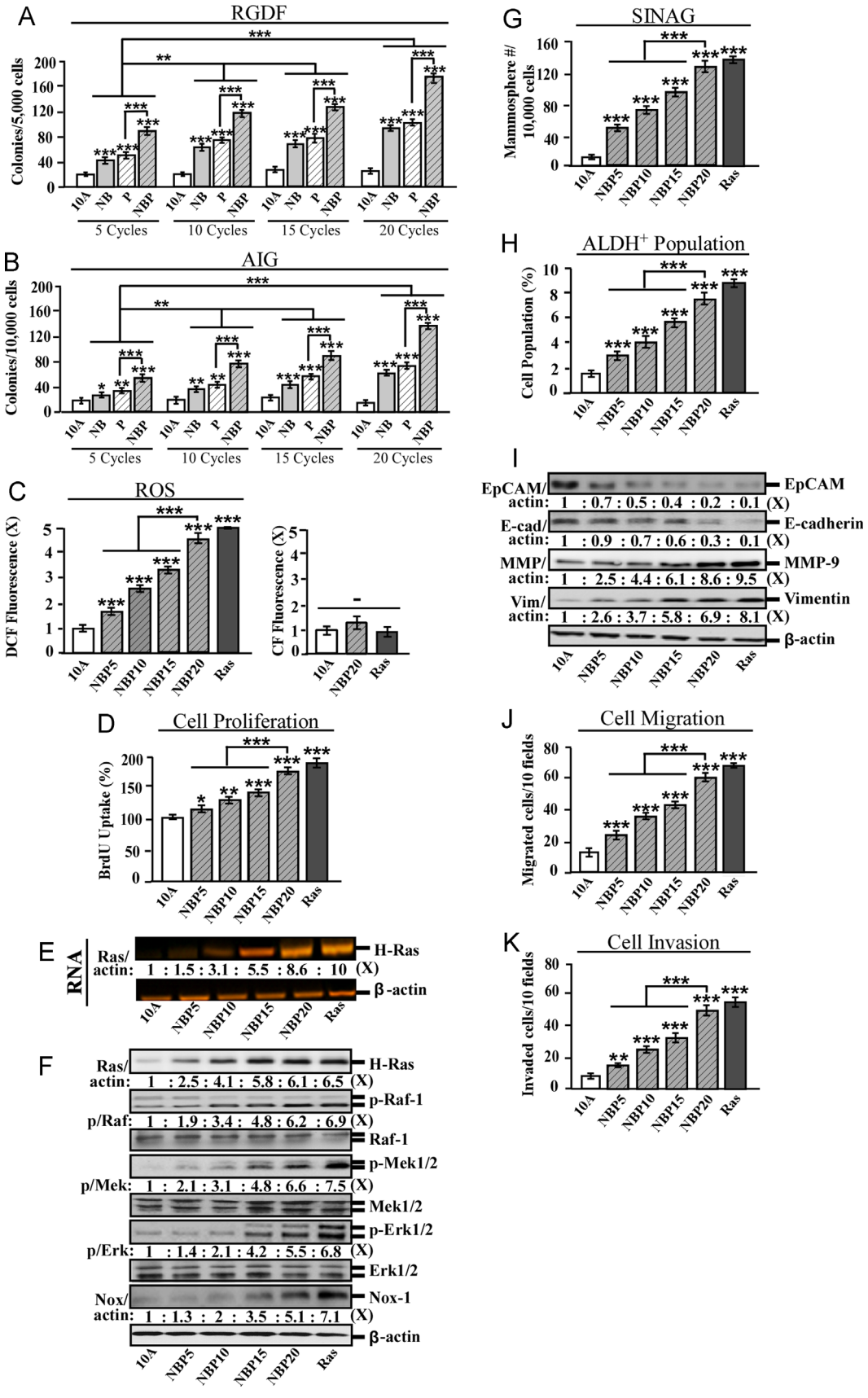
Introduction

Breast cancer is the most common type of cancer and second leading cause of cancer-related death among North American and European women [1]. Most breast cancers occur sporadically due to chronic exposure to multiple environmental carcinogens; this multistep process results in the transformation of breast cells from noncancerous to precancerous and then to cancerous [2,3]. We have developed a breast cell carcinogenesis model to mimic sporadic breast cancer development associated with long-term exposure to low doses of carcinogens [4–9]. In this model, we repeatedly expose immortalized, noncancerous, human breast epithelial cells to physiologically achievable doses of carcinogens to progressively induce cellular acquisition of various cancer-associated properties

Abbreviations: 5(6)-CFDA, 5-(and-6)-carboxyfluorescein diacetate; AIG, anchorage-independent growth; ALDH, aldehyde dehydrogenase; ATCC, American Type Culture Collection; B[α]P, benzo[α]pyrene; BrdU, 5-bromo-2′-deoxyuridine; CF, carboxyfluorescein; CM medium, complete MCF10A medium; CM-H_2DCF-DA, chloromethyl-dichlorodihydrofluorescein-diacetate; DCF, dichlorodihydrofluorescein; DEAB, diethylaminobenzaldehyde; ER, estrogen receptor; ELISA, enzyme-linked immunosorbent assay; EMT, epithelial-to-mesenchymal transition; EpCAM, epithelial cell adhesion molecule; Fpg, formamidopyrimidine (fapy)–DNA glycosylase; LM medium, low-mitogen medium; MMP-9, matrix metalloproteinase-9; MTT, methyl thiazolyl tetrazolium; NAC, *N*-acetyl-*L*-cysteine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; Nox, NADPH oxidase; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5–b]pyridine; RDGF, reduced dependence on growth factors; ROS, reactive oxygen species; NB, NNK plus B[α]P; NBP, NB plus PhIP.

* Corresponding author at: Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, 2407 River Drive, Knoxville, TN 37996, USA. Fax: +1 865 974 5640.
E-mail address: hcrwang@utk.edu (H.-C. Wang).

http://dx.doi.org/10.1016/j.freeradbiomed.2014.12.015
0891-5849/


A
RGDF
Colonies/5,000 cells
10A NB P NBP
5 Cycles
10 Cycles
15 Cycles
20 Cycles
B
AIG
Colonies/10,000 cells
C
ROS
DCF Fluorescence (X)
CF Fluorescence (X)
10A NBP5 NBP10 NBP15 NBP20 Ras
D
Cell Proliferation
BrdU Uptake (%)
E
RNA
Ras/
actin: 1 : 1.5 : 3.1 : 5.5 : 8.6 : 10 (X)
H-Ras
β-actin
F
Ras/
actin: 1 : 2.5 : 4.1 : 5.8 : 6.1 : 6.5 (X)
H-Ras
p-Raf-1
p/Raf: 1 : 1.9 : 3.4 : 4.8 : 6.2 : 6.9 (X)
Raf-1
p-Mek1/2
p/Mek: 1 : 2.1 : 3.1 : 4.8 : 6.6 : 7.5 (X)
Mek1/2
p-Erk1/2
p/Erk: 1 : 1.4 : 2.1 : 4.2 : 5.5 : 6.8 (X)
Erk1/2
Nox/
actin: 1 : 1.3 : 2 : 3.5 : 5.1 : 7.1 (X)
Nox-1
β-actin
G
SINAG
Mammosphere #/ 10,000 cells
H
ALDH+ Population
Cell Population (%)
I
EpCAM/
actin: 1 : 0.7 : 0.5 : 0.4 : 0.2 : 0.1 (X)
EpCAM
E-cad/
actin: 1 : 0.9 : 0.7 : 0.6 : 0.3 : 0.1 (X)
E-cadherin
MMP/
actin: 1 : 2.5 : 4.4 : 6.1 : 8.6 : 9.5 (X)
MMP-9
Vim/
actin: 1 : 2.6 : 3.7 : 5.8 : 6.9 : 8.1 (X)
Vimentin
β-actin
J
Cell Migration
Migrated cells/10 fields
K
Cell Invasion
Invaded cells/10 fields