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HRAS G13R transformation effect on MCF10A cells harboring different p53 variants

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Master's Thesis

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Dedication

To my mother and father...

To my wife

To my brothers and sisters...

To my friends...

Ahmad Mousa Ismael Jaffal

Declaration

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this thesis has not been submitted for higher degree to any other university or institution.

Signed: .....

Derar Sameeh Abdel-Aziz Khader

Date: 21/12/2019

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List of abbreviations

°C	Degree Celsius
Bax	Bacl-2 associated X protein
BCL-2	B-cell lymphoma 2
cDNA	Complementary DNA
cm	centimeter
CGS	Cancer related gene signature
COSMIC	Catalogue of somatic mutations in cancer
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
EMT	Epithelial mesenchymal transition
EMC	Extracellular matrix
ER	Estrogen receptor
ERBB2	v-erb-b2 avian erythroblastic leukemia oncogene homolog 2
g	gram
h	hour
HER2	Human epidermal receptor 2
KO	Knockout
L	liter
M	Molar
MDM2	Mouse double minute 2 homolog
mg	Milligram

min	Minute
mL	Milliliter
mRNA	Messenger RNA
ng	Nanogram
nm	Nanometer
nM	Nanomolar
nt	Nucleotide
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PI3K	Phosphoinositide 3-kinase
rpm	Round per minute
Rb	Retinoblastoma
RNA	Ribonucleic acid
SFM	Serum free media
Sec	Second
TCGA	The cancer genome atlas
TP53	Tumor protein 53
U	Units
TP53 WT	Wilde-type *TP53* gene
TP53 Wtres	Wild-type rescue *TP53* gene
TP53 R173H	*TP53* gene with *R173H* mutation
TP53 R273H	*TP53* gene with *R273H* mutation
TP53 KO	knocked out *TP53* gene
µL	Microliter

μM	Micromolar
Fig	Figure
MCF10A Wtress	MCF10A cell that wild-type *TP53* gene is knocked out from them and overexpress wild-type *TP53* gene
MCF10A R175H	MCF10A cell that wild-type *TP53* gene is knocked out from them and *TP53* gene with R175H mutation
MCF10A R273H	MCF10A cell that wild-type *TP53* gene is knocked out from them and *TP53* gene with R273H mutation
MCF10A KO	MCF10A cell that wild-type *TP53* gene is knocked out from them
MCF10A WT	Normal MCF10A cells with wild-type *TP53* gene

Abstract

Breast cancer is the most common cancer and the leading cause of death among women worldwide. Mainly imbalance between tumor suppressor genes and oncogenes lead to cancer transformation. This imbalance usually arises from mutations in one or more of either tumor suppressor genes or oncogenes. Different gene expression patterns among breast cancer subtypes lead to heterogeneity and give different phenotypes. Our preliminary data showed that different *TP53* variants resulted in different gene expression patterns. So we hypothesized here that combinations between *HRAS G13R* and different *TP53* variants will lead to different gene expression patterns and different phenotypes. To test this hypothesis, we infected MCF10A cell harboring different *TP53* variants (*TP53 KO, TP53 R175H, and TP53 R273H*) with *HRAS G13R* viral vector. Afterward we tested proliferation, migration, survival, and apoptotic resistance of manipulated cells. In addition, we tested some of phenotypic related target genes expression. Our results showed that *HRAS G13R* overexpression increases tumorigenicity of infected cells with *HRAS G13R-TP53 R175H* combination having highest tumorigenic effect. Also results of different tested assays shows that cell proliferation, migration, survival, and resistance to apoptosis was affected differentially in each of *HRAS G13R* and *TP53* variants combination. This phenotypic diversity was combined with difference in gene expression patterns between different combinations. Overall our study provides a new model that spots the light on the role of two hit system in cancer transformation and progression. In addition, this model may help in understanding *TP53* and HRAS crosstalk in breast cancer and help in cancer diagnosis and treatment.

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Chapter 1

1. Introduction

1.1. Cancer transformation

Cancer is a generic term for a large group of diseases characterized by the growth of abnormal cells beyond their usual boundaries that can then invade adjoining parts of the body and/or spread to other organs. It accounts for 9.6 million deaths worldwide in 2018 (WHO, 2019). Many factors are responsible for cancer development and progression. All these factors lead to the main cause of cancer which is the imbalance between tumor suppressor genes and oncogenes. This imbalance can be either due to mutations in tumor suppressor genes, oncogenes or in DNA repair genes (Osborne et al., 2004). The involvement of epigenetics in this process of cancer development added more complexity to understating cancer initiation and progression mechanisms (Wu et al., 2015). So in order to make progression in cancer diagnosis and therapy, greater understanding of cancer molecular mechanisms is needed on both direct gene alterations and epigenetic alterations (Hinshelwood and Clark, 2008). During cancer transformation, a lot of phenotypic properties related to transformed cells are uncovered, these properties are called cancer hallmarks and are described as major hallmarks that in part include self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (Hanahan and Weinberg, 2011).

1.2. Breast Cancer

Breast cancer is the leader cause of female cancer related deaths worldwide. It's responsible for more than half million deaths among women in 2018 (15% of cancer deaths), and yearly there are about 2 million new cases of breast cancer are reported among women (WHO, Breast Cancer 2019). Several factors affect breast cancer prognosis and survival rate. Those factors include age, ethnic group, hormones, and genetic factors (Libson and Lippman, 2014). Breast cancer is a very heterogeneous type of cancer which can be classified into different categories based on breast cancer type,

appearance of the tissue, stage of cancer, and gene profile (cancer genome Atlas 2012). Huge efforts are targeted toward developing strategies to fight this cancer starting with early detection of disease and not limited to surgical procedures, radiotherapy, chemotherapy and biological and targeted therapy (Libson and Lippman, 2014). Targeted therapy is very important due to heterogeneity of the disease (Sousa et al., 2019). This heterogeneity makes the war against breast cancer more complicated, increase treatment cost in addition to decrease the chances of treatment availability to vast group of patients (especially in low-income and middle-income countries were individual therapy is not always provided) (Jamison et al., 2015). For instance, not all HER2 positive patient's respond to trastuzumab (a drug targeted toward HER2 receptor) in the same way, and survival rate are different among them. This thought to be underlined by several resistance mechanisms including heterodimerization, with other HER receptors, and bypassing HER2 signaling pathways (Baselga et al., 2012). These resistance mechanisms of cancer made researchers focus towards developing new strategies to overcome resistance mechanisms and prevent recurrence of disease (Luo et al., 2015). Moreover, it has been determined that within single breast carcinoma there are multiple cancer cell clones, harboring distinct genetic and epigenetic profiles (Sousa et al., 2019). This intra tumor heterogeneity is highly affected by tumor micro-environment (McGranahan and Swanton, 2017, Colak and Medema, 2014). Due to the mentioned reasons, standard therapies against breast cancer are not enough and do not prevent cancer recurrence (Sousa et al., 2019), and more studies focusing on different underlying molecular mechanisms of different breast cancer categories are needed.

1.3. The Oncogene-RAS

RAS genes are one of the earliest oncogenes discovered in human tumors. This family includes *K-RAS, HRAS,* and *N-RAS* (Downward, 2003). The protein products of RAS proto-oncogenes family are a group of small GTPases that play vital role in signal transduction through numerous growth factors to stimulate cell proliferation and movement. *RAS* proto-oncogene is frequently mutated in cancers and affects a variety of processes involved in cancer progression (Pylayeva-Gupta et al., 2011, Kiaris and Spandidos, 1995). Mutations in *RAS* genes were found in about 30% of all human cancers(Adjei, 2001). The mutations in *RAS* proteins make the protein product constituently active due to unresponsiveness to GTPase activating protein (GAPs) (Downward, 2003).