hTR Regulation Of Telomerase Activity Under Hypoxic Condition

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Antecedents / State Of The Art

Telomeres , telomerase and cancer

Telomeres are repetitive sequences of nucleotides at the ends of each chromosome in order to prevent them for degradation during replication.

Human telomerase is a ribonucleoprotein composed of a catalytic protein subunit with reverse transcriptase activity (hTERT) and a template-containing RNA component (hTR). Its function is to synthesize multiple tandem repeats of telomeric DNA, which confer stability to telomeres length.

Telomerase is active in germ line cells and becomes deactivated during differentiation. Oncogenically transformed cells that lack telomerase activity can bypass senescence but then die during crisis. However, cancer cells with active telomerase can continue to proliferate and become unafected by senescence (fig.1).

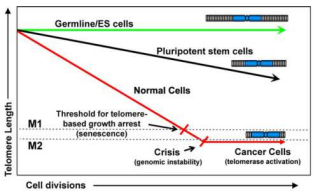


Figure 1. The telomere hypothesis for cellular mortality. Figure from Shay JW. *et al* (2011).

Hypoxia and tumor progression

The majority of human solid tumors contain regions of hypoxia, which is caused by reduced oxygen delivery to the cells. This sets off a series of events that take advantage of normal cellular stress response machinery intended to relieve cells of their stressful environment through altered gene expression.

Transcriptional regulation by hypoxia-inducible factor-1 (HIF-1α) represents the most important mechanism mediating adaptative response to a O_2-reduced environment.

A link between hypoxia and telomerase regulation

Previous studies have demonstrated telomerase activity up-regulation under hypoxic conditions in solid tumors (ovarian carcinoma and colon adenocarcinoma).

Transcriptional activation of *hTR* and *hTERT* gene promoters under hypoxia is caused by HIF union to consensus hypoxia response element (HRE) and other regulation mechanisms.

General Aims

1. To investigate the role and regulation of human telomerase RNA (hTR) in basal-like and claudin-low breast carcinoma cell lines cultured under hypoxic condition.
2. To assess the role of hypoxia along hTR regulation and tumoral transformation of non-carcinogenic breast cell lines

Specific Aims / Expected Results

1 **To quantify telomerase activity in breast cancer cell lines cultured under hypoxia.**

Expected results: Increase in telomerase activity caused by up-regulation of telomerase genes.

2 **To identify in vitro mechanisms that regulate *hTR* transcription levels under hypoxic conditions.**

2.1 **To identify the presence of regulatory mechanisms of *hTR* transcriptional activation by HIF-1α.**

2.2 **To identify the presence of other regulatory mechanisms of hTR (e.g. chromatin remodeling).**

Expected results: 2.1. HIF-1α union to HRE element in *hTR* gene promoter as a transcriptional regulator under hypoxia. 2.2. Existence of other regulation mechanisms, such as chromatin changes in *hTR* sequence..

3 **To establish the maximum concentration of oxygen that causes up-regulation of *hTR* in different breast cancer cell lines. To relate results obtained in each cell line with its differential features.**

Expected results: It is expected to obtain similar results in cell lines of each molecular kind, while there might be differences between basal-like and claudin-low used cell lines because of their differential features.

4 **To detect telomerase activation in non-carcinogenic breast cell lines under hypoxic conditions.**

4.1 **If so, to identify regulation mechanisms involved in this activation, analyzing *hTR* expression specifically.**

Expected results: Apoptosis resistance and cellular transformation of normal breast cancer cell lines due to telomerase activation caused by hypoxia.

Material And Methods

Stress treatments

Use of cell lines (table 1):
- MDA-MB-468, BT20, MDA-MB-231 and HS578T for aims 1-3.
- MCF10A for aim 4.

NORMOXIA — Standard conditions

HYPOXIA — 1%, 2%, 5% and 10% O_2

Table 1. Molecular features of breast cancer cell lines chosen for this study.

Cell line	Classification	ER	PR	HER2
MCF10A	Non-tumorigenic	-	-	
MDA-MB-468	Basal	-	-	
BT20	Basal	-	-	
MDA-MB-231	Claudin-low	-	-	
HS578T	Claudin-low	-	-	

ER, estrogen receptor; PR, progesterone receptor; HER2; epidermal growth factor 2. ER/PR/HER2 status: ER/PR positivity (+ positive; - negative); HER2 overexpression (+)

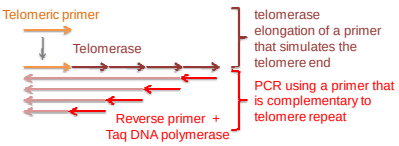
Measurement of telomerase activity

Aims 1 and 4

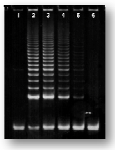
PROTEIN EXTRACTION

Negative control: inactivation of telomerase by heat denaturation

Telomeric Repeats Amplification Assay
TRAPeze® Telomerase Detection Kit



12.5 % polyacrylamide gel electrophoresis + imaging techniques

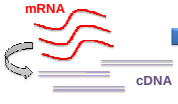


Quantification *of hTR* transcripts

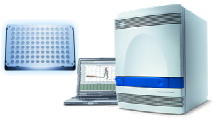
Aims 2-4

RNA EXTRACTION

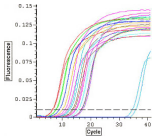
RT- PCR



Real-time PCR



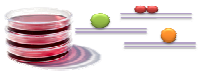
hTR mRNA quantification



Identification of *hTR* transcriptional activation by HIF-1α or chromatin remodeling mechanisms

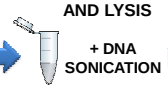
Aims 2 and 4

DNA-protein CROSSLINKS



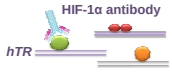
10 minutes in 1% neutral buffered formaldehyde

NUCLEI EXTRACTION AND LYSIS + DNA SONICATION



200-600 bp fragments

ChIP



Real-time PCR

Negative control: sample with no antibody
Also with antibodies for specific histone modifications

Project Benefits And Social Impact

- Hypoxic condition in tumors is crucial for tumor progression and leads to
  - Therapy resistance
  - Aggressive clinical course
  - Poor patient outcome
- Basal like and claudin-low breast cancer cell lines growth cannot be inhibited by neither anti-estrogen therapy or trastusumab (used as breast cancer treatment)

Need to improve treatments

- **↑Telomerase activity during tumor development**
- Telomerase inhibitors as adjuvant therapy to improve the efficiency of chemotherapeutic agents.

Study of hTR regulation mechanisms in this cell lines and condition might be useful for developing a new therapeutic approach.

Dissemination of findings

Research findings
- Better understanding of telomerase regulation mechanisms.
- Interesting for therapeutic approaches development

Scientific community
- Research findings will be shared with:
  - a) Researchers
  - b) Higher education

Results communication
- Conferences and meetings
- Publications in scientific journals, newsletters and websites.

References:
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