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Oestrogen Receptor Mutations And Their Influence on Breast Cancer Growth.

Karin Dagmar Amoils

A thesis submitted to the Faculty of Medicine, University of Johannesburg, in fulfilment of the requirements for the Doctor of Philosophy.

Plettenberg Bay, 2011

Declaration

I declare that this thesis is my own, unaided work. It ha
before for any degree or examination at any other Universit
in this thesis was done while I was employed by the Department
University of the Witwatersrand.

_____

K.D. AMOILS

__day__ _____ __2011.

This research was approved by the Committee for Research
University of the Witwatersrand. (Human Ethics Clearance N

For

my beautiful daughter

Shannon

and my very patient husband

Johnny

All rising to great places is by a winding stair.

Buddha

ABSTRACT

Oestrogen receptor (ER) mutations have been identified fo
previous studies. The effects of the deletion variants due
clinical parameters, prognosis and treatment in 61 breast
carcinoma patients and 13 control samples from elective re
procedures, respectively. RNA extracted from fine needle
breast tissue was reverse transcribed and using nested
analysis the presence of these variants established. Using
tests their significance with respect to clinical parameters s
nodal involvement, stage, presence or absence of metasta
and hormone responsiveness was determined. Kaplan-Meier
also determined.

The MCF-7D breast cancer cell line was cloned with two clone
further analysis, namely TCA3 (hormone sensitive) and TCC
These clones were treated for ten passages with 17β-oestroge
oestradiol and oestriol; oestrogen precursors, androstenedi
anti-oestrogen, 4-hydroxytamoxifen; and the aromatase in
aminoglutethimide, respectively. RNA was extracted from
after the tenth passage and ERα and ERβ exon profiles were exam
RT-PCR and sequence analysis. After the tenth passage hor
were performed every 24 hours (up to 96 hours) with
determined using the MTT assay.

The results indicate that ER± variants do not have any affect
to menstrual status and nodal involvement (N). Expression
required by the mouse monoclonal® antibody 1D5 (DAKO
immunocytochemical assay used for the recognition of
assess ER status and therefore show significance. ER±"
previous investigations, the variant ER±"3 were not foun
tumourigenesis. ER±"5 was observed to be more prevale
patients and was usually expressed with the complete ER±5 i
heterodimerization. ER±"5 showed no significance with res
disease or response to hormone treatment.

An increase in the ratio of type ER±"4 to ER±4 indicated an increa
metastatic potential of diseased tissue. ER±4 and ER±"4
present in MCF7D clones and after 10 passages the TCA3 c
10^{-8}M aminoglutethimide indicated a complete loss of ER±
hormone responsiveness. Results suggest that ER±"4 may pla
progression of disease but not in the acquisition of tamoxif

ER±"6 was observed in 15% of patient tumour cytosols and in the MCF7D control
samples. An increase in the expression of ER±"6 in breast cancer samples
significantly increased their metastatic potential ($p = 0.018$). ER±"6 was also
observed as significant with respect to oestrogen independent growth ($p = 0.023$), indicating the
possible relevance of ER±"6 in progression of the disease.

ER±"7 was the most frequently observed variant and did no
significance with regard to any of the clinical parameters e
of ER±"7 did not show significance with regard to hormone
vitro the presence of this variant, as a heterodimer with the w
ER±7, conferred greater sensitivity to tamoxifen in the ta
TCC1.

Multiple exon deletions of ER± were also observed. The
multiple deletion variants were ER±"4, namely ER±"2
and ER±"6. The multiple variant ER±"6 may be involved in
tumour progression.

ER² variants were not examined in as much detail as ER
insufficient material available for analysis. The binding doma
domain and the ligand binding domain, of ER² were analy
patients and in 47 cell lines. They were not found to be sig
respect to the clinical parameters investigated and the ER
and TCC1 lines remained unchanged after 10 passages unde
conditions.

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CONFERENCE PRESENTATION

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K. D. Amoils, P. MacPhail, P. Ruff and R. Duarte.

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LIST OF ABBREVIATIONS

2n	diploid
4n	tetraploid
aa	amino acids
AF	activation function
AI	aromatase inhibitor
AMV	avian myeloblastosis virus
ATCC	American Tissue Culture Collection
bp	base pairs
^{o}C	degrees centigrade
cDNA	complementary deoxyribonucleic acid
CO_2	carbon dioxide
CoR	coregulator
DBD	DNA binding domain
DEPC	diethylene pyrocarbonate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbent assay
EMEM	Earle s Minimal Essential Medium
ER	oestrogen receptor
ER±	oestrogen receptor alpha

ER²	oestrogen receptor beta
ERE	oestrogen response element
ERICA	oestrogen receptor immunocytochemical a
FCS	foetal calf serum
FNA	fine needle aspirate
FSH	follicle stimulating hormon
h	hours
HCl	hydrogen chloride
hGR	human glucocorticoid receptor
HPRT	hypoxanthine phosphoribosyltransferase
HRT	hormone replacement therapy
hsp	heat shock protein
IGFR	insulin like growth factor receptor
I.U.	international unit
kb	kilobase
KCl	potassium chloride
kDa	kiloDalton
LBD	ligand binding domain
LH	luteinizing hormone
M	molar
M-phase	mitotic phase
MA	megestrol acetate
MAPK	mitogen activated protein kin

$MgCl_2$	Magnesium chloride
min	minute
MISS	membrane-initiated steroid signaling
ml	millilitre
mM	millimolar
MMP	matrix metalloproteinase
MPA	medroxyprogesterone acetate
miRNA	microRNA
mRNA	messenger ribonucleic acid
MTT	3-4,5 dimethyl-2h,5acid-2,5 diphenyl tetrazolium bromide
N	nodal involvement
NA	norethisterone acetate
NaOH	sodium hydroxide
ng	nanograms
NH_2	amino
NISS	nuclear-initiated steroid signaling
oes	oestradiol
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pmol	picomole
PR	progesterone receptor
PRPP	phosphoribosyl pyrophosphate

RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RPMI-1640	Roswell Park Memorial Institute Medium 1640
RT-PCR	reverse transcribed polymerase chain reaction
s	seconds
SERM	selective oestrogen receptor modulator
SFCS	stripped foetal calf serum
SPF	S-phase fraction
STAR	Study of Tamoxifen and Raloxifene
T	tumour size
T_m	melting temperature
TAE	Tri-acetate EDTA
tamox	tamoxifen
Taq	Thermus aquaticus
TD	touchdown
TDLU	terminal ductal lobular units
TGF-α	transforming growth factor alpha
USF	upstream stimulatory factor
UTR	untranslated region
UV	ultraviolet
V	volts
wt	wild type