EGF AND PDGF RECEPTORS IN ENDOMETRIAL CANCER

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ABSTRACT

Despite its relatively good long term survival compared to other cancers in the female, endometrial carcinoma still kills 20% of those women who develop the disease worldwide. Abnormalities in growth factor receptors have been shown to be important in both the prognosis and probably the malignant transformation of the two other oestrogen-dependent cancers, breast and ovary, which suggests that similar defects may occur in endometrial cancer.

In this thesis, two of these growth factor receptors, those for the epidermal and platelet-derived growth factors, are studied in endometrial cancer at the levels of the gene and expression of the proteins on the cell surface, in an attempt to detect abnormalities which might be implicated in carcinogenesis.

No amplification or rearrangement of the EGFR gene was detected on 13 tumour samples initially, or of the EGF, PDGF α- and β-subunit receptor genes subsequently on a mixture of tumour samples and cell from endometrial cancer cell lines.

The size of the EGF and PDGF receptor proteins and the activation of their tyrosine kinase domains was assessed in an oestrogen responsive (Ishikawa) and an oestrogen independent (HEC-1-A) endometrial cancer cell line. Functioning receptors for EGF were demonstrated in both cell lines but no PDGF receptors were detected. EGF-binding studies were carried out and appropriate affinity constants and receptor numbers obtained in both cell lines.

The growth of Ishikawa and HEC-1-A cells in culture was studied but because of the rapid growth of both lines in the absence of serum no meaningful mitogenic effects were shown by any growth factor or steroid.

Phorbol ester and TGF-β inhibited the growth of HEC-1-A and Ishikawa cells, respectively. Of particular interest was that, despite the reported sensitivity of Ishikawa cells to oestrogen, no consistent stimulation was observed.

The growth of both cell lines in the absence of serum suggested that the cells might be secreting growth factors and conditioned medium was obtained from long term, large scale cultures. The medium was concentrated and passed through a size exclusion column and the fractions assayed for DNA synthesis and competition for EGF-binding to Swiss 3T3 cells. No consistent effect on either of these assays was shown and no further investigation was undertaken.

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LIST OF ABBREVIATIONS

A_{280}	absorbance at 280 nm
αTPA	alpha form of TPA
APC	adenomatous polyposis coli gene
APS	ammonium persulphate
bFGF	basic FGF
BES	N,N-bis(2-Hydroxyethyl)-2-aminoethanesulfonic acid
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
CCM	concentrated, conditioned medium
cDNA	complementary DNA
cm	centimetre
CM	conditioned medium
cpm	counts per minute
CSF	colony stimulating factor
CSF-1	colony stimulating factor-1
CSF-1R	colony stimulating factor-1 receptor
DAG	1,2-diacylglycerol
dATP	deoxyadenosine triphosphate
DC	direct current; dextran:charcoal
DCC	deleted in colon cancer gene
DC-FCS	dextran:charcoal-stripped FCS
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
DMEM	Dulbecco's modified Eagle's medium
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
DTT	dithiothreitol
dTTP	deoxythymidine triphosphate
EDTA	ethylenediamine tetra-acetic acid
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ER	oestrogen receptor
FAP	familial adenomatous polyposis
FCS	foetal calf serum
FGF	fibroblast growth factor

FIGO	International Federation of Gynaecologists and Obstetricians
FPLC™	fast protein liquid chromatography
g	gram
G1,2,3	grade 1,2,3; first growth phase of cell cycle
GAP	GTPase activating protein
GFR	growth factor receptor
GDP	guanosine diphosphate
GTP	guanosine triphosphate
h	hour
HCl	hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
ICI 164,384	N-n-butyl-11-3,17β-dihydroxyoestra-1,3,5(10)-trien-7α-yl-N-methylundecamide
^{125}I-EGF	^{125}I-labelled EGF
IGF-1	insulin-like growth factor-1
IGF-1R	insulin-like growth factor-1 receptor
IL-3	interleukin-3
IP_3	1,4,5-triphosphate
kb	kilobase
kD	kilodalton
l	litre
LICR	Ludwig Institute for Cancer Research
LOH	loss of heterozygosity
M	mole, molar
MCC	mutated in colon cancer gene
µg	microgram
µl	microlitre
mg	milligram
min	minute
ml	millilitre
mM	millimolar
mRNA	messenger RNA
MW	molecular weight
ng	nanogram
nM	nanomolar
NRK	normal rat kidney

$NR6^+$	subclone of Swiss 3T3 cells with transfected EGFR
OCs	oral contraceptives
OD	optical density
4-OHtam	trans 4-hydroxytamoxifen
O-2A	oligodendrocyte-type 2 astrocyte
p	short arm of chromosome
PBS	phosphate-buffered saline
PDGF	platelet-derived growth factor
PDGF-αR	PDGF alpha receptor subunit
PDGF-βR	PDGF beta receptor subunit
PDGFR	PDGF receptor
pg	picogram
PI	phosphatidylinositide
PI3-K	phosphatidylinositol 3'-kinase
PKC	protein kinase C
PLC-γ	phospholipase C-γ
pM	picomolar
PMSF	phenylmethylsulfonyl fluoride
PR	progesterone receptor
PTK	protein-tyrosine kinase
q	long arm of chromosome
R5020	promegestone
RB	retinoblastoma (gene/protein)
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute
RR	relative risk
RU486	17β-hydroxy-11β-(4-dimethylamino-phenyl)-17α-(1-propynyl)-estra-4,9-dien-3-one
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SEM	standard error of mean
SSC	standard saline citrate
SSV	simian sarcoma virus
TAE	tris-acetate EDTA
TE	tris-EDTA

TEMED	N,N,N',N'-tetramethylethylenediamine
TGF-α	transforming growth factor-α
TGF-β	transforming growth factor-β
tris	tris(hydroxymethyl) amino methane
TPA	phorbol 12-tetradecanoate 13-acetate
UV	ultraviolet
v-*onc*	viral oncogene
v/v	volume for volume
WT1	Wilms tumour gene
w/v	weight for volume