Biomedicine & Pharmacotherapy 85 (2017) 399–411

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Research article

Estrogen and thyroid cancer is a stem affair: A preliminary study

Mariangela Zane[a], Carmelo Parello[a], Gianmaria Pennelli[b], Danyelle M. Townsend[c], Stefano Merigliano[a], Marco Boscaro[d], Antonio Toniato[a], Giovannella Baggio[e], Maria Rosa Pelizzo[a], Domenico Rubello[f,*], Isabella Merante Boschin[a]

[a] Department of Surgical, Oncological, and Gastroenterological Sciences, University of Padova, Padova, Italy
[b] Surgical Pathology and Cytopathology Unit, Department of Medicine, University of Padova, Padova, Italy
[c] Department of Drug Discovery and Pharmaceutical Sciences, Medical University of South Carolina, USA
[d] Endocrinology, Department of Medicine, University of Padova, Padova, Italy
[e] Internal Medicine Unit, Department of Molecular Medicine, University of Padua, Padova, Italy
[f] Santa Maria della Misericordia Hospital, Rovigo, Italy

ARTICLE INFO

Article history:
Received 16 August 2016
Received in revised form 10 November 2016
Accepted 11 November 2016

Keywords:
Estrogen
Thyroid cancer
Cancer stem cells
Gender medicine
Cancer signaling

ABSTRACT

Gender influences Papillary Thyroid Cancer (PTC) with an incidence of 3:1 when comparing women to men with different aggressiveness. This gender discrepancy suggests some role of sex hormones in favoring the malignant progression of thyroid tissue to cancer. Estrogens are known to promote Stem Cell self-renewal and, therefore, may be involved in tumor initiation. The goals of these studies are to investigate the underlying causes of gender differences in PTC by studying the specific role of estrogens on tumor cells and their involvement within the Cancer Stem Cell (CSC) compartment. Exposure to 1 nmol l^{-1} Estradiol for 24 h promotes growth and maintenance of PTC Stem Cells, while inducing dose-dependent cellular proliferation and differentiation following Estradiol administration. Whereas mimicking a condition of hormonal imbalance led to an opposite phenotype compared to a continuous treatment. *In vivo* we find that Estradiol promotes motility and tumorigenicity of CSCs. Estradiol-treated mice inoculated with Thyroid Cancer Stem Cell-enriched cells developed larger tumor masses than control mice. Furthermore, Estradiol-pretreated Cancer Stem cells migrated to distant organs, while untreated cells remained circumscribed. We also find that the biological response elicited by estrogens on Papillary Thyroid Cancer in women differed from men in pathways mediated. This could explain the gender imbalance in tumor incidence and development and could be useful to develop gender specific treatment of (PTC).

1. Introduction

Gender bias occurs across a wide-variety of seemingly unrelated diseases. From a clinical management standpoint, why do various pathologies affect women and men in a differently with respect to incidence, progression and clinical outcomes? This phenomenon could be easily explained for pathologies involving gender specific reproductive organs, but not those disorders originating in organs, such as Thyroid gland, which are common to both. The goal of these studies is to focus on gender differences that impact the thyroid gland with specific focus on cancer.

Thyroid cancer (TC) incidence is on the rise worldwide. In Italy it is the second most common cancer in women, after breast cancer, and the fifth most common in men [8]. Specifically, Papillary Thyroid Cancer (PTC) incidence is three times higher in women compare to men. Moreover, women are more likely to be affected at the beginning of the reproductive age, with a peak between 40 and 49 years, whereas men are affected later in life, around at 60–69 years and have a lower disease-free survival [17]. Whilst the principal causes of TC development, such as nutritional factors (i.e., Iodine uptake), ionized radiation and genetic changes in BRAF, RET, and NTRK, seem not to be involved in this gender discrepancy [17] some studies have reported a correlation between the number of ovulatory cycles, high number of pregnancies, and lactation suppressant and TC incidence [5,25]. Furthermore, other studies have demonstrated that long exposure to exogenous estrogens is associated with the occurrence of TC [1,6,21]. Collectively, these studies suggest a specific role for sex hormones, and in particular

* Corresponding author at: Santa Maria della Misericordia Hospital, Via Tre Martiri 140, 45100 Rovigo, Italy.
E-mail address: Domenico.rubello@libero.it (D. Rubello).

http://dx.doi.org/10.1016/j.biopha.2016.11.043
0753-3322/

Nomenclature

Acronyms and abbreviations

ABCB5	ATP binding cassette subfamily B member 5
ABCG2	ATP binding cassette subfamily G member 2
ACTB	Actin beta
ALDH (ALDH1A1)	Aldehyde dehydrogenase 1 family member A1
ANGPT1	Angiopoietin 1
ANGPT2	Angiopoietin 2
AURKA	Aurora kinase A
B2M	Beta-2-microglobulin
BETA-CATENIN	Catenin beta-1
BMP	Bone morphogenetic protein
BRAF	B-Raf proto-oncogene, serine/threonine kinase
BSA	Bovine Serum Albumins
CCND2	Cyclin D2
CCND3	Cyclin D3
CDC20	Cell division cycle 20
CSC	Cancer stem cell
DACH1	Dachshund family transcription factor 1
DDIT3	DNA damage inducible transcript 3
DEAB	Diethylaminobenzaldehyde
DKC1	Dyskerin pseudouridine synthase 1
DLL1	Delta like canonical Notch ligand 1
DMEM	Dulbecco's Modified Eagle Medium
DNMT1	DNA methyltransferase 1
DUOX1	Dual oxidase 1
E2	Estradiol
EGF	Epidermal growth factor
EMT	Epithelial-Mesenchymal Transition
ERs	Estrogen Receptors
ERA	Estrogen Receptor Alpha
ERB	Estrogen Receptor Beta
ERBB3	Erb-b2 receptor tyrosine kinase 3
FBS	Fetal bovine serum
FGF2	Fibroblast growth factor 2
FGFR2	Fibroblast growth factor receptor 2
GADD45	Growth arrest and DNA damage inducible alpha
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GATA3	GATA binding protein 3
GPER1	G protein-coupled estrogen receptor 1
H&E	Haematoxylin and eosin
ID1	Inhibitor of DNA binding 1, HLH protein
IL8	Interleukin-8
ITGA6	Integrin subunit alpha 6
JAG1	Jagged 1
KLF17	Kruppel like factor 17
LATS1	Large tumor suppressor kinase 1
LIN28A	Lin-28 homolog A
LIN28B	Lin-28 homolog B
MAML1	Mastermind like transcriptional coactivator 1
MKI67	Marker of proliferation Ki-67
NANOG	Homeobox protein NANOG
NIS	Sodium/iodide cotransporter
NOD/SCID	Non-obese diabetic/severe combined immunodeficiency
NOTCH1	Neurogenic locus notch homolog protein 1
NTRK	High affinity nerve growth factor receptor
OCLN	Occludin
OCT3	POU class 5 homeobox 1
PAX8	Paired box 8
PBS	Phosphate buffered saline
PECAM1	Platelet and endothelial cell adhesion molecule 1
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PPP1R15A	Protein phosphatase 1 regulatory subunit 15A
PRLP0	Ribosomal protein lateral stalk subunit P0
PTC	Papillary Thyroid Cancer
PTCH1	Patched 1
RET	Ret proto-oncogene
SCM	Stem cell medium
SERPINF1	Pigment epithelium-derived factor
SKP2	S-phase kinase associated protein 2
SNAI1	Snail family transcriptional repressor 1
SMO	Smoothened, frizzled class receptor
SOX2	SRY-box 2
STMN1	Stathmin 1
TAZ	Tafazzin
TC	Thyroid Cancer
TEP1	Telomerase associated protein 1
TG	Thyroglobulin
TGFB	Transforming growth factor beta-1
TPO	Thyroid peroxidase
TSHR	Thyroid stimulating hormone receptor
TTF1	Transcription termination factor 1
TWIST1	Twist family bHLH transcription factor 1
TWIST2	Twist family bHLH transcription factor 2
UICC	Union for International Cancer Control
VEGFA	Vascular endothelial growth factor A
VEGFR1	Vascular endothelial growth factor receptor 1
VEGFR2	Vascular endothelial growth factor receptor 2
WEE1	WEE1 G2 checkpoint kinase
ZEB1	Zinc finger E-box binding homeobox 1

for Estrogen, in regulating thyroid function. In recent years different researchers have begun to examine the estrogen role in the development of thyroid pathologies [9,28]. Estrogen is known to be involved in cellular processes such as growth, cell motility and organ function. Consistent with this, different research groups have reported Estrogen in the modulation of TC proliferation and migration [10,12,15,18,23,30]. Estradiol (E2) is the most potent form of estrogen being that it has the highest affinity to its receptors ERα, ERβ, and GPER1 [4,19]. In particular, ERα stimulates proliferation with an anti-apoptosis effect, while ERβ is associated with apoptosis and growth inhibition. For this reason, the ERα/ERβ ratio is helpful to elucidate the TC pathophysiology [13,19]. Studies in mice have demonstrated that circulating estrogens are directly responsible for increased susceptibility of female mice to thyroid disease. Specifically, E2 activate PI3K pathway, inhibit p27, and affect the transcriptional regulation of thyroid genes (i.e., TPO, DUOX1, and NIS) [3]. Despite this and other studies demonstrating a strong direct effect by estrogens on thyroid growth and function, the specific dynamics that move the development and the initiation of proliferative and neoplastic disorders still remains to be clarified.