Molecular and Cellular Endocrinology xxx (2015) 1–10

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Mouse models of thyroid cancer: A 2015 update

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ARTICLE INFO

Article history:
Received 10 April 2015
Received in revised form
28 May 2015
Accepted 11 June 2015
Available online xxx

Keywords:
Thyroid cancer
Mouse modeling
Genetics
Pre-clinical models

ABSTRACT

Thyroid cancer is the most common endocrine neoplasm, and its rate is rising at an alarming pace. Thus, there is a compelling need to develop *in vivo* models which will not only enable the confirmation of the oncogenic potential of driver genes, but also point the way towards the development of new therapeutics. Over the past 20 years, techniques for the generation of mouse models of human diseases have progressed substantially, accompanied by parallel advances in the genetics and genomics of human tumors. This convergence has enabled the development of mouse lines carrying mutations in the genes that cause thyroid cancers of all subtypes, including differentiated papillary and follicular thyroid cancers, poorly differentiated/anaplastic cancers, and medullary thyroid cancers. In this review, we will discuss the state of the art of mouse modeling of thyroid cancer, with the eventual goal of providing insight into tumor biology and treatment.

1. Introduction

Thyroid carcinoma is the most common endocrine malignancy. It affects about 1% of the population (Nikiforova and Nikiforov, 2008) and its incidence is on the rise across the globe and in United States, particularly in women (Jemal et al., 2008). Although modern imaging techniques including ultrasonography, CT scanning, and nuclear imaging provide significantly enhanced detection of thyroid lesions, the rise in incidence appears to be greater than would be predicted solely from improved case finding (Enewold et al., 2009).

The thyroid itself is composed of epithelial cells (thyrocytes), which form the thyroid follicles and secrete thyroid hormone, and C-cells, neuroendocrine-derived cells which inhabit the interfollicular spaces and secrete calcitonin. Cancers can arise from either cell type, although epithelial thyroid cancers make up 95–97% of human thyroid cancer. Epithelial thyroid cancers (also referred to as non-medullary thyroid cancers, NMTC) are divided into three types based on histology: papillary, follicular, and anaplastic. Of these subtypes, papillary thyroid cancer (PTC) is the most common, comprising about 80% of all thyroid cancers. Follicular thyroid cancer (FTC) accounts for another 10–15%, and anaplastic thyroid cancer (ATC) makes up to about 5%. The remaining few percent are medullary thyroid cancers (MTC).

Although the overall survival of patients with localized thyroid cancer is >95% at 5 years, there is a subset of patients who develop metastatic disease. The prognosis of these patients with metastatic thyroid cancer of any histopathologic subtype drops significantly, as thyroid cancers respond poorly to systemic therapy. By historical measures, the use of RAI can be considered the first example of 'targeted' therapy, but patients that are not cured by initial surgery followed by an appropriate RAI dose typically are not cured by subsequent doses. In the new era of targeted therapies, there has been significant excitement about the development of tyrosine kinase inhibitors targeted to the molecular abnormalities in thyroid (and other) cancer subtypes. In practice, these treatments have provided significant benefit, but the time of response tends to be self-limited. Thus, there is a pressing need for the development of new therapies which will be effective in the patient arena.

In the past, much of the work on new drug development relied on the use of cell line models, and there are well-established human cell line models for each of the major thyroid cancer subtypes. Drug studies can be performed *in vitro* in the tissue culture lab, or can be performed *in vivo* using xenografts into immunosuppressed mice. Traditional techniques have used subcutaneous tumor implants (Kim et al., 2005), although recent efforts to develop more physiologic systems have described the use of eutopic implants of

Abbreviations: PTC, Papillary thyroid cancer; MTC, Medullary thyroid cancer; FTC, Follicular thyroid cancer; RAI, radioactive iodine; TCGA, The Cancer Genome Atlas; Tg, Thyroglobulin; TSH, Thyroid stimulating hormone; MEN2, Multiple Endocrine Neoplasia, type 2.

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http://dx.doi.org/10.1016/j.mce.2015.06.029
0303-7207/

thyroid cancer cells into the thyroid bed (Kim et al., 2005, Nucera et al., 2009). As a model of metastasis, thyroid cancer cells have also been injected into the tail vein or into the cardiac vasculature to produce widespread tumors (Li et al., 2013, Zhang et al., 2014). However, the limited effectiveness of agents which work well in these models points to the need to develop new therapies based on tumors which arise *in situ* in the immune competent host. To this end, there has been substantial interest over the past few years to develop genetically engineered mouse models for thyroid cancer. Not only do these models provide an *in vivo* confirmation of the genetic drivers of thyroid cancer, but they also provide an optimal setting for pre-clinical testing of new drug treatment paradigms. In the age of personalized medicine, it has been possible to generate mouse lines with the most common human mutations driving thyroid cancer. If a drug can be effective at treating a cancer in such a model system, there would then be a much stronger expectation that the drug would be effective in the corresponding patients, an approach supported by early data (Chakravarty et al., 2011, Ho et al., 2013).

In this review, we will discuss the current state-of-the-art in the development of genetically engineered mouse models for human thyroid cancer. While traditional mouse modeling has relied on the production of transgenic lines by pro-nuclear DNA injection into blastocysts (Hanahan et al., 2007) and of knockout lines (either conventional or conditional) by homologous recombination in mouse embryonic stem (ES) cells (Capecchi, 1989), newer technologies have expanded the repertoire of possible models. The techniques include the introduction of cre-inducible alleles (using a so-called lox-STOP-lox cassette) (Soriano, 1999), the use of inducible exogenous genes (generally using a tetracycline/doxycycline inducible or suppressible system) (Schonig et al., 2010) and drug-inducible cre activity (generally the Cre-ERT2 transgene, which is activated by tamoxifen treatment) (Indra et al., 1999). In addition, the capabilities of genome editing techniques such as TALENs (Hermann et al., 2014), zinc-finger nucleases (Sung et al., 2012), and the CRISPR/Cas9 system (Wang et al., 2013) are only beginning to be explored. Thus, although mouse modeling has reached a certain level of sophistication, the field should continue to evolve rapidly.

For now, a current survey of mouse models for NMTC resulting from single gene mutations and multi-gene mutations are presented in Tables 1 and 2, respectively, and models for MTC are presented in Table 3. The details of these models are discussed in the text. However, it is worthwhile recalling that once alleles are generated, they can be crossed to other mice in order to study tumor promotion or suppression. The sheer number of potential crosses makes fully cataloging all published crosses an endeavor which would only serve to cloud the value of the information presented. Thus, although we have tried to describe the major thyroid cancer models, this review is not meant to be an exhaustive list of all mice which have thyroid cancer as part of their phenotype. The key consideration is that as these models and the tools with which to analyze them become more sophisticated, it is expected that their value as pre-clinical models for therapeutic evaluation will continue to grow. Eventually, these mice should enable the development of new therapies which will improve our ability to treat patients with aggressive forms of thyroid cancer.

Table 2
Multi-hit models for NMTC in the mouse.[a]

Gene 1	Gene 2	Phenotype
PPFP TX[b]	*Pten* KO	Metastatic FTC
K-Ras^{G12D} KI	*Pten* KO	Metastatic FTC
Prkar1a KO	*Pten* KO	Metastatic FTC
THRB$^{PV/PV}$	*K-Ras* KI	ATC
RET-PTC1 TX	*Tp53* KO	ATC
RET-PTC3 TX	*Tp53* KO	ATC
BRAF^{V600E} TX	*Tp53* KO	ATC
Pten KO	*Tp53* KO	ATC
BRAF^{V600E} TX	*PIK3CA*^{H1047R} *KI-I*	ATC

[a] References for each of the models can be found in the text.
[b] Abbreviations as in Table 1.

2. Mouse models of papillary thyroid cancer

2.1. Phenotype of human PTC

Papillary thyroid cancer (PTCs) are typically unencapsulated tumors characterized in humans by papillary architecture and a specific nuclear feature called “nuclear grooves”, which are easily noted by experienced thyroid pathologists. These tumors frequently exhibit overlapping nuclei with ground-glass appearance and invaginations of cytoplasm into the nuclei (Schlumberger,

Table 1
Single gene models for non-medullary thyroid cancer in the mouse.[a]

Gene	Mutation	Human phenotype	Mouse phenotype	Alleles[b]
BRAF	V600E	PTC (usually cPTC or fvPTC)	PTC	TX KI KI-I TX-I
RET-PTC1	Fusion gene	PTC	PTC (non-invasive)	TX
RET-PTC3	Fusion gene	PTC	PTC (non-invasive)	TX
TRK-T1	Fusion gene	PTC	PTC (non-invasive)	TX
H-RAS	G12D/G12V	PTC or FTC	hyperplasia	TX/KI
K-RAS	G12D	PTC or FTC	hyperplasia	TX/KI
N-RAS	Q61K	PTC or FTC	PTC/FTC	TX
Rap1b	G12V	ND	hyperplasia	KI-I
PAX8-PPARG	Fusion gene	FTC	hyperplasia	TX
PTEN	Knockout	FTC or PTC	FTC (likely strain dependent)	Het KO
Prkar1a	Knockout	FTC or PTC	FTC	Het KO
THRB$^{PV/PV}$	PV (fs443)	RTH (het)[c]	FTC	KI (homo)
PIK3CA	H1047R	FTC (?)	Minimal hyperplasia	KI-I
SV40 T-ag	Viral gene	–	ATC	TX

[a] References for each of the models can be found in the text.
[b] TX: Transgenic, KI: Knock-in, KI-I: Inducible Knock-in, TX-I: Inducible transgenic, Het: Conventional heterozygote, KO: Knockout (tissue-specific).
[c] RTH: Resistance to thyroid hormone. Note that patients are heterozygous for the mutation. The phenotype is observed in homozygous mice.

Please cite this article in press as: Kirschner, L.S., et al., Mouse models of thyroid cancer: A 2015 update, Molecular and Cellular Endocrinology (2015), http://dx.doi.org/10.1016/j.mce.2015.06.029