Thyroid Follicular and C cells in Preclinical Toxicology

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Thyroid Gland

- Largest endocrine gland
- Devoted only to endocrine function
- Oldest gland phylogenically
  - All vertebrates, many invertebrates

NIS IHC: 2 mo. old Mouse

NIS IHC: 9 mo. old Mouse
Short Term Response of Follicular Cells To Increased TSH Secretion

Long Term Response of Follicular Cells To Increased TSH Secretion

Hypertrophy and Hyperplasia of Follicular cells (Goiter)

Goiter

- Major human health problem
- Predominantly due to iodine deficiency
- Estimated over 200 million people affected
  - Adults
- Iodine deficient regions
  - E.g., Great Lakes in USA

Goiter

- Ancient disease
  - Described for over 5000 years
- Historical interpretation
  - Sign of beauty
  - Punishment of gods
Goiter: Outcome

- Human
  - Diffuse Hyperplasia

- Rat
  - Diffuse hyperplasia
  - Focal hyperplasia
  - Neoplasia

- Dog
  - Diffuse hyperplasia

Goiter: Causes

- Deficient iodine intake
- Goitrogenic chemicals
- Genetic enzyme defects
- Iodine excess

Rat Thyroid Gland Tumorigenesis

**Mechanisms to Disrupt Thyroid Function**

- **Direct Thyroid Effect**
  - Inhibit Hormone Synthesis
  - Inhibit iodide uptake; inhibit TPO
  - Inhibit Hormone Secretion
  - Follicular Cell Cytotoxicity

- **Peripheral Effect**
  - Competition of thyroid hormone binding proteins
  - Inhibition of $T_4$ deiodination
  - Increased metabolism and clearance of $T_4$ or $T_3$

Thyroid Follicular Neoplasia

**Humans**

- Thyroid cancer: Most common endocrine malignancy
  - Incidence has risen in past 4 decades
  - Uncommon deaths
- Thyroid nodules are common
  - Palpable: 4-7% of adults
  - Ultrasound: up to 67%, usually women
  - Most are benign
  - 5-15% are malignant
Human Relevance Framework

Rat Thyroid Follicular Tumors

- Fundamental differences in thyroid hormone economy in rats
  - Rapid half-life of T₄
  - Lack of thyroid binding globulin
  - High TSH concentrations (greater in males)
  - Low secretion rate of T₄ (inherently less able to make T₄ compared to humans)
  - Sensitive to the tumorigenic effects of drugs that decrease T₄ or T₃
  - Robust TSH response decreased T₄ or T₃

Human Relevancy Framework

Thyroid Hormone Economy

- Rats (esp. males) have increased incidence of proliferative lesions compared to humans likely due to increased TSH concentrations
  - Male rats have higher TSH compared to females
- Rats have shorter half-life of T₄ (12-24 h) vs. 5-9 days in humans
  - Due to less high affinity binding globulin, TBG, in rats
- Rats require greater T₄ (20 µg/kg) compared to humans (2.2 µg/kg) to substitute for the thyroid gland

Rat Thyroid Gland

Thyroxine (T₄)

Serum Protein Binding

<table>
<thead>
<tr>
<th>Species</th>
<th>TBG</th>
<th>Postalbumin</th>
<th>Albumin</th>
<th>Prealbumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Monkey</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Dog</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Mouse</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Rat</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Chicken</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
</tbody>
</table>

TBG: Thyroxine (T₄) Binding Globulin
Prealbumin (Transthyretin, TTR)

Drug-Induced Tumors in Rodents
(order of prevalence)

- RATS
  - Thyroid
  - Liver
  - Testis
  - Mammary Gland
  - Adrenal
  - Pituitary

- MICE
  - Liver
  - Lung
  - Mammary Gland
  - Blood
  - Ovary
Tumorigenic Drugs in Rats (1)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Product Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone</td>
<td>Antiarrhythmic</td>
</tr>
<tr>
<td>Atenolol</td>
<td>β-Adrenergic Blocker</td>
</tr>
<tr>
<td>Bepridil</td>
<td>Ca-Channel Blocker</td>
</tr>
<tr>
<td>Dapsone</td>
<td>Antineoplastic</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>Antibiotic</td>
</tr>
<tr>
<td>Iodinated Glycerol</td>
<td>Expectorant</td>
</tr>
<tr>
<td>Methimazole</td>
<td>Anti-Thyroid</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Sedative</td>
</tr>
</tbody>
</table>

Tumorigenic Drugs in Rats (2)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Product Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenobarbital</td>
<td>Antiepileptic</td>
</tr>
<tr>
<td>Minocycline</td>
<td>Antibiotic</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>Antianxiety</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>Ca-Channel Blocker</td>
</tr>
<tr>
<td>Sertraline</td>
<td>Antidepressant</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>Hypolipidemic</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>Diuretic</td>
</tr>
<tr>
<td>Vidarabine</td>
<td>Antiviral</td>
</tr>
</tbody>
</table>

Follicular Adenoma: Rat

Follicular Carcinoma: Rat

Inhibition of Hormone Synthesis

<table>
<thead>
<tr>
<th>Inhibition of Iodide Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Competition for NaI Symporter (NIS)</td>
</tr>
<tr>
<td>– Thiocyanate</td>
</tr>
<tr>
<td>– Perchlorate (ClO₄⁻)</td>
</tr>
<tr>
<td>• Rats more sensitive than humans, mice, rabbits</td>
</tr>
</tbody>
</table>

Inhibition of Thyroperoxidase (TPO)

<table>
<thead>
<tr>
<th>Inhibition of Thyroperoxidase (TPO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Organification of I₂ to tyrosine and coupling of iodotyrosines</td>
</tr>
<tr>
<td>– Thiourea: reduces I₂ to I⁻</td>
</tr>
<tr>
<td>• Inhibition of TPO</td>
</tr>
<tr>
<td>– Thioamides</td>
</tr>
<tr>
<td>• Propylthiouracil</td>
</tr>
<tr>
<td>• Mercaptoimidazole</td>
</tr>
<tr>
<td>• Methimazole, carbimazole, aminotriazole</td>
</tr>
<tr>
<td>• Sulfonamides, such as sulfamethazine</td>
</tr>
<tr>
<td>– Sulfonylureas (antidiabetic drugs)</td>
</tr>
<tr>
<td>– 1st generation: acetohexamide, chlorpropamide, tolbutamide, tolazamide</td>
</tr>
<tr>
<td>– Substituted phenols</td>
</tr>
<tr>
<td>• Resorcinol, salicylamide</td>
</tr>
</tbody>
</table>
Species Sensitivity to TPO inhibition by Sulfonamides

<table>
<thead>
<tr>
<th>Sensitive Species</th>
<th>Resistant Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Humans</td>
</tr>
<tr>
<td>Mouse</td>
<td>Primates</td>
</tr>
<tr>
<td>Dog</td>
<td>Guinea pig</td>
</tr>
<tr>
<td>Pig</td>
<td>Chicken</td>
</tr>
</tbody>
</table>

Inhibition of Hormone Secretion

*Excess of Iodide, Lithium*

- Excess of iodide
  - Decreased lysosomal proteases (humans)
  - Inhibition of colloid droplet formation (rats, mice)
  - Inhibition of TSH-mediated cAMP (dogs)
  - Excessive maternal intake of iodine
    - Goiter in offspring not adult
- Lithium
  - Inhibits colloid droplet formation by cAMP
  - Inhibits hormone release

Competition for Thyroid Hormone Binding Proteins

- Less important in species with TBG
- Binding to prealbumin (transthyretin)
  - Chlorophenols, chlorophenoxy acids, nitrophenols
- Decreased $T_4$ in rats
  - Pentachlorophenol, 2,4-dichlorophenoxyacetic acid (2,4-D), dimethox, and bromoxynil
- Decreased $T_3$ in rats
- Decreased $T_4$ and $T_3$ in rats
  - 2,4-D

Thyroid Hormone Deiodination

*Activation and Metabolism*

- $3,3',5'-T_4$ to $3,3',5'-rT_3$
- $3,5',3'-T_3$
- $3',5'-rT_3$
- $3,3'T_2$

*5' (outer ring) - Deiodinase 2*

- $5'D_2$
**Thyroid Hormone Deiodination**

*Activation and Metabolism*

- Deiodinase 1 (liver, kidney, thyroid)
  - Outer and inner ring deiodination
  - Substrates: rT<sub>3</sub> → T<sub>4</sub>, T<sub>3</sub>
  - Inhibited by propylthiouracil
  - Stimulated by T<sub>3</sub>
- (5')-Deiodinase 2 (brain, pituitary, placenta, thyroid, skeletal muscle, brown fat)
  - Outer ring deiodination only
  - Major activating enzyme
  - Substrates: T<sub>4</sub> → rT<sub>3</sub>
- Deiodinase 3 (brain, pregnant uterus, fetus, placenta)
  - Inner ring deiodination
  - Substrates: T<sub>3</sub> > T<sub>4</sub>

**Inhibition of Thyroxine (T<sub>4</sub>) Deiodination**

- T<sub>4</sub> functions as a prohormone
- Selenium deficiency
  - Se: cofactor for type I 5'-monodeiodinase
  - Lack of Se leads to decreased T<sub>3</sub> and increased T<sub>4</sub>
- FD&C Red No. 3 and iopanoic acid
  - Inhibits type I 5'-monodeiodinase
  - Rats: Increased T<sub>4</sub>, decreased T<sub>3</sub>, increased reverse T<sub>3</sub>, increased TSH
- Lipid peroxidation
  - Type I 5'-monodeiodinase

**UDP-GT**

*Uridine 5'-diphospho-glucuronosyltransferase*

- Important Phase II conjugative enzyme
  - Elimination of drugs and foreign chemicals
  - Not present in cats
- Transfers glucuronol from uridine 5'-diphospho-glucuronic acid to lipophilic substrates with O, N, S, or carboxyl groups
- Increases water solubility for renal excretion
Inducers of UDP-GT

**Examples**

- Phenobarbital (PB)
- Pregnenolone-16α-carbonitrile (PCN)
- 3-methylcholanthrene (3MC)
- Arochlor 1254 (PCB)

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**Effects of Microsomal Enzyme Inducers in Rats**

<table>
<thead>
<tr>
<th>Compound</th>
<th>PB</th>
<th>PCN</th>
<th>3MC</th>
<th>PCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_4$-UDP-GT</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>$T_3$-UDP-GT</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Serum $T_4$</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Serum $T_3$</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Serum TSH</td>
<td>↑</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Thyroid Cell</td>
<td>↑</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
</tr>
</tbody>
</table>

Thyroid Cell Proliferation

CD Klaassen, Tox. Pathol., 2001

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**Endocrine Disrupters (EDCs) Thyroid Gland**

- Endocrine Disrupter Chemicals
  - (anti)-estrogenic compounds
  - (anti)-androgenic compounds
  - Interference with steroidogenesis
- Dysregulation of thyroid hormones
  - Similar mechanisms as preclinical drug toxicity in rats
  - Assays
    - Rats (28-day, pubertal, adult male)
    - Amphibian Metamorphosis Assay

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**Amphibian Metamorphosis Assay**

- Detect substances that interact with hypothalamic-pituitary-axis during development
  - Conserved thyroid structure in vertebrates
- Metamorphosis of *Xenopus Laevis* (African Clawed Frog) tadpoles
- Test for Endocrine Active Substances (EAS)
- OECD (Org. Economic Co-operation & Development guidelines)
  (www.oecd.org/dataoecd/44/52/40909207.pdf)
Nieuwkoop and Faber (N&F) Stages

- 21-day test
  - Hind limb length, development stage, thyroid histology
- Nieuwkoop and Faber (N&F) staging
  - 45-49: First form tadpole
  - 49-56: Second form tadpole
  - 56-60+: Third form tadpole

Human Relevance Framework

Thyroid Follicular Tumors: MOA & Key Events

↓ T4 or T3 (many mechanisms)

↓ Inhibition of thyrotropes in pituitary

↑ TSH secretion, thyro trope hyperplasia

Thyroid follicular cell hypertrophy

Colloid depletion

Increased cell proliferation

Hyperplasia

Mouse: TSH Cell Hyperplasia
(chemical inhibition of thyroxine synthesis)
Human Relevance Framework

*Thyroid Follicular Tumors: MOA & Key Events*

- Hyperplasia
- Adenoma
  - Carcinoma (death)
  - Metastasis (death)

Calcitonin

Calcitonin Synthesis and Secretion

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**Pathophysiology of Calcitonin Mammals**

- No clinical conditions associated with calcitonin excess or deficiency
  - **Biomarker:** C-cell hyperplasia, tumors
  - Drug Tx: Hypercalcemia, osteoporosis

**Calcitonin Content in Endocrine Glands**

<table>
<thead>
<tr>
<th>Gland</th>
<th>Calcitonin Activity (MRC units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utimobranchial</td>
<td>4000-6000</td>
</tr>
<tr>
<td>Salmon</td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>100-200</td>
</tr>
<tr>
<td>Pig</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td></td>
</tr>
</tbody>
</table>

**Therapeutic Uses of Calcitonin**

- Osteoporosis
- Analgesia in vertebral fractures
- Hypercalcemia - short lived effect

Salmon calcitonin
100 times more potent than human calcitonin

**C-Cell Numbers**

- Humans
  - < 1% of thyroid cells
  - May decrease with age
- Rats
  - 5% of thyroid cells
  - Increase to 10% after 120 days
  - Individual cells (intrafollicular and parafollicular)
  - Interfollicular clusters
    - increase in number and size with age

**C-Cell Distribution: Rat Thyroid Gland**

Martin-Lacave, Cell Tissue Res, 1992
Thyroid C-Cell Tumors

Species Occurrence

- Rat (F344, SD, WAG/Rij)
  - Higher incidence in females (Wistar)
  - OVX decreases CT synthesis and secretion
- Bull (ultimobranchial)
- Dog
- Mouse
- Horse
- Ferret (with islet cell tumors)
- Moufflon (sheep)
- Zebrafish (ultimobranchial)

Factors Influencing Development

Thyroid C-Cell
Proliferative Lesions

- Irradiation
- Vitamin D
- High Dietary Calcium

Thyroid C-Cell Proliferative Lesions

Diffuse C-Cell Hyperplasia

Focal C-Cell Hyperplasia
C-Cell Carcinoma

C-Cell Histopathology: Mice

- Use immunohistochemistry
  - Normal C-cells
  - Diffuse hyperplasia
  - Early focal hyperplasia

- Can only see focal hyperplasia and tumors in routine histopathology

Mouse: Normal

Diffuse Hyperplasia
### Tumorigenic Drugs in Rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Product Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exenatide</td>
<td>GLP-1R agonist (F)</td>
</tr>
<tr>
<td>Liraglutide</td>
<td>GLP-1R agonist (M/F)</td>
</tr>
<tr>
<td>Aledronate</td>
<td>Bisphosphonate (M)</td>
</tr>
<tr>
<td>Arformoterol</td>
<td>$\beta_2$ receptor agonist (F)</td>
</tr>
<tr>
<td>Atenolol</td>
<td>$\beta_2$ receptor agonist (M)</td>
</tr>
<tr>
<td>Colesevelam</td>
<td>Bile acid sequestrant (F)</td>
</tr>
<tr>
<td>Naratriptan</td>
<td>5-HT$_{1D/1B}$ receptor antagonist (M/F)</td>
</tr>
<tr>
<td>Palonosetron</td>
<td>5-HT$_3$ receptor antagonist (F)</td>
</tr>
</tbody>
</table>

### Tumorigenic Drugs in Mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Product Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liraglutide</td>
<td>GLP-1R Agonist (M/F)</td>
</tr>
</tbody>
</table>

(likely class effect)

### Glucose Homeostasis

**Fasting vs. Postprandial**

- **Fasting glucose level**
  - Insulin
  - Glucagon
- **Postprandial glucose level**
  - Incretin (GI) hormones
    - GLP-1 (glucagon-like peptide-1)
    - GIP (gastric inhibitory polypeptide; glucose-dependent insulinotropic polypeptide)
    - Only GLP-1 increases insulin in diabetics

### GLP-1

**Very Short Half-Life in Blood**

- Half-life: 1-5 minutes
- GLP-1 (7-36) amide and 7-37 forms
- Degraded by plasma DPP-4 (dipeptidyl peptidase-4)
  - DPP-4 inhibitors: Limited ability to increase GLP-1

### Calcitonin Secretion in Mice

Knudsen et al, Endocrinology, 2010
**Exenatide (Byetta®)**

- First GLP-1 agonist (2005)
- Synthetic form of exendin-4
  - Isolated from Gila monster salivary glands
  - 50% homology to human GLP-1
  - Longer half-life in humans
- Adjunctive therapy for DM
- Twice daily injections

**Long-Acting GLP-1 Agonists**

- Liraglutide (Victoza®)
  - Modified rGLP-7-37 with palmitic acid moiety
  - Once daily injection
- Bydureon®
  - Exenatide and microsphere formulation
  - Once weekly injection
- Dulaglutide (once weekly)
  - GLP (7-37) linked to Fc IgG fragment
- Albiglutide (once weekly or biweekly)
  - GLP-1 dimer fused to albumin
- Lixisenatide (once daily)

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**C-cells and GLP-1 agonists**

*Rats and Mice*

- C-cell hyperplasia
  - Diffuse
  - Focal
- C-cell adenomas
- C-cell carcinomas
- Rats are more sensitive than mice

**Human Relevance Framework**

*C-cell Tumors in Rodents: Mode of Action & Key Events*

- Drug binding to GLP-1R on C-cells
  - ↑ Cytoplasmic cAMP & CT secretion
  - ↑ C-cell proliferation
  - ↑ C-cell diffuse hyperplasia
  - ↑ C-cell focal hyperplasia
  - ↑ C-cell adenomas
  - ↑ C-cell carcinomas

---

**GLP-1R in Rodent C-cells**

*Weight of Evidence Approach*

- Immunohistochemistry
- In situ hybridization
- Receptor binding in vivo
- Downstream effects in rodent C-cell lines and not in human C-cell lines
- Lack of findings in dogs and NHP
- Lack of downstream and proliferative effects in GLP-1R KO mice

**GLP-1 Agonist-Induced C-cell Proliferation in Rodents**

- Rodent-specific effect (?)
- Receptor expression greatest in rodents
- GLP-1R KO mice confirm role of receptor
  - Physiologic role in rodents
- No proliferation in dogs and NHP
- No increase in calcitonin in dogs and NHP
- Equivocal or no increase in calcitonin in humans
- *Long-term effect in humans need to be monitored*