Photodiagnosis/therapy for specific treatment of ovarian cancer

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Presentation Plan

• Introduction ovarian cancer and photomedicine
• Photodetection
• Therapy
• Current research
• Conclusion / Perspective
Figure 1

*Common sites of ovarian cancer metastases.*

'Ovarian cancer spreads fast to the whole abdominal cavity by exfoliation.'
Epithelial Ovarian Cancer

- Fourth most frequent cause of “cancer-related” death
- 65% diagnosed with stage III-IV disease
- 80% chemo-sensitive (initial response)
- 5 year survival rate: 15-20%
- 50% of “cured” patients (negative second look laparotomy) will recur
“The facts remain that a large number of patients are being treated almost to the point of “cure” and an additional stroke of some sort is needed.”

(DiSaia, Clinical Gynecological Oncology, Mosby-Year Book, 1997)
Photodynamic Principle

- Use of a photo-enhancing or photosensitizing chemical to aid in the diagnosis or treatment of a target cell
Photophysical Processes

Fluorescence detection

Photodynamic Therapy

Fluorescence

 ISC

τ = 1ns

IC

400 nm

630 nm

Absorption

630 nm

700 nm

Phosphorescence

τ = 10µs

Singlet Oxygen production

Collision energy transfer

S0

S1

S2

τ = 250 ns

Δ d = 45 nm

Spectroscopy

1 * O2

3 O2

Energy
PHOTORADIATION THERAPY OF CANCER
(Laser–Hematoporphyrin Derivative)

Cancer

48-72 hours

Drug selectively retained by cancer cells

Inject HPD (drug) in vein

Fiber optic bundle

Argon Laser

Dye Laser

514 nm
488 nm
(Blue-Green Light)

625-635 nm
(Red Light)

$^{1}\text{O}_2$ Kills Cells

sens + hv $\rightarrow$ 1 sens*

1 sens* $\rightarrow$ 3 sens*

3 sens* + 3 $\text{O}_2$ $\rightarrow$ $\text{O}_2$ + sens

$\text{O}_2$ + substrate $\rightarrow$ oxidation

sens = HPD
Photosensitizers

• Porphyrins
  – Photofrin (PF)
  – "Aminolevulinic acid (ALA)", Protoporphyrin IX (PpIX)

• Chlorins
  – m-Tetrahydroxyphenyl chlorin (mTHPC)
  – Benzoporphyrin derivative mono-acid (BPD)
  – Tin ethyl etiopurpurin (SnET2)

• Phtalocyananines
Haem Biosynthesis

5-ALA synthase

Uptake of exogenous 5-ALA

PBG Deaminase

Cytoplasm

Lower rate in tumour cells

Coproporphyrinogen III

Uroporphyrinogen III

Porphyrinogen

Feedback control

Mitochondria

Fe

Fe^{2+}

Protoporphyrinogen IX

Protoporphyrin IX

Glycine

5-ALA

Cycline + succinyl CoA

Ferrochelatase

Lower rate in tumour cells

Higher rate in tumour cells
AIMS

• To evaluate photodetection of ovarian cancer peritoneal implants in the animal model
• To evaluate photodetection of ovarian cancer peritoneal implants in patients
• To analyse toxicity of ALA photodynamic therapy (PDT) in the animal model
**NuTu-19 Ovarian Cancer Animal Model**

- Completely analogous to human epithelial ovarian cancer.
- **Cell line** - NuTu-19 - Spontaneous mutation.
- **Histology** - Poorly differentiated ovarian adenocarcinoma with papillary features.
- **Growth pattern** - I.P. serosal nodules with local tissue invasion (omentum, diaphragm, liver, peritoneum).
- **Malignant ascites** - average vol. 50-70ml in 6 weeks.
- **Survival** - 10^6 cells I.P are 100% fatal; mean survival of 50 days.
- **Non-immunogenic tumor developed in an immunocompetent host.**

Rose et al AJOG 9/96
Light micrographs (A) and fluorescence (B) of a peritoneal nodule (size < 0.5 mm) 6 hr after ip ALA administration. Magnification (C) of the peritoneal serosa (boxed area in B) showing a thin layer of tumor matching with the fluorescence

PHOTODETECTION
Epithelial ovarian cancer PDD in NuTu-19 rat model

8mM h-ALA IV prior to photodetection 2 hours later

Ludicke F et al, Br J Cancer 2003
Human Epithelial Ovarian cancer PDD

10mg/ml ALA applied topically prior to photodetection

PHOTODYNAMIC THERAPY

Dye (PS) → Photons → Activated Dye → $O_2$ → Toxicity
CONCLUSIONS

• Photodetection has been shown to be efficient in the animal model and feasible in patients

• Photodetection of ovarian cancer peritoneal implants, not visible by other methods, is a conceivable goal for the future

• ALA-PDT did not succeed in our animal model
Phototherapy for specific treatment of ovarian cancer
Haem Biosynthesis

5-ALA synthase

Uptake of exogenous 5-ALA

PBG Deaminase

Cytoplasm

Lower rate in tumour cells

Higher rate in tumour cells

Ferrochelatase

feedback control

Mitochondria

Fe^{2+}

Protoporphyrin IX

Protoporphyrinogen IX

Uroporphyrinogen III

Coproporphyrinogen III

Cytoplasm

Glycine

5-ALA

5-ALA

Porphyrinogen

Coproporphyrinogen III

Uroporphyrinogen III

Protoporphyrinogen IX

Protoporphyrin IX

5-ALA

5-ALA

5-ALA

5-ALA

5-ALA

5-ALA
Issues in gene therapy

- Vectors (plasmid, virus, nanoparticle)
- Side effects
- Tissue penetration
- Immune reaction
- Specificity
AIM

• Proof of principle of photodynamic therapy of the peritoneal cavity.

STRATEGY

• Establishment of a stable NuTu 19 ALA-S cell line with a doxycyclin ON system:
NuTu-19 treated with 5’ALA

Bright field

PDT filter
ALA-synthase-NuTu-19

Bright field

PDT filter
CONCLUSIONS

• Efficient Pp IX production and PDT effects after application of ALA-S virus (CMV) on normal NuTu 19 cells

• Good PpIX production in ALA-S NuTu cells after doxycyclin application

• Efficient photodynamic therapy of ALA-S NuTu 19 ovarian cancer cells after doxycyclin application
Perspective

- Establishment of the ALA-S NuTu 19 ovarian cancer model
- Proof of efficient photodynamic therapy in the animal model after doxycyclin administration, impact on survival
- Studies with different vectors and promoters
- Achieve cancer specific expression of the transgene
COLOR PLATE 1. Transfer of genes to pleural mesothelial cells after intrapleural administration of an adenovirus gene transfer vector encoding an intracellular protein. The lungs and diaphragm were harvested 3 days after right intrapleural or, for comparison, intratracheal and intravenous administration of 109 PFU of an Ad vector encoding β-galactosidase (Adβgal) to BALB/c mice. Control animals received 100 ml of phosphate-buffered saline by the intrapleural route. All sections were stained with the X-Gal reagent and counterstained with nuclear fast red; a blue color indicates cells expressing β-galactosidase activity. (A–E) Lung tissue. (A) Right intrapleural administration of PBS as control. (B) Right intrapleural administration of Adβgal. (C) Intratracheal administration of Adβgal. (D) Intravenous administration of Adβgal. (E) Intravenous administration of 3.3 × 10^5 CT26.CL25 tumor cells expressing β-Gal as control to demonstrate β-Gal activity within the vascular compartment. (F) Right diaphragm from the same animal as in (B). Magnification bar: 50 μm. Mae et al, Hum Gene Ther 2002.
A photosensitising adenovirus for photodynamic therapy

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