AGING-BIOTECHNOLOGY RELATIONSHIP

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Aging and biotechnology relationship

Introduction

- Aging is a natural process
- Senescent cells replaced with juvenile
- Senescence appears in different levels of tissue, organ, cell, and molecule

Senescence, derived from senex meaning "old man"
Introduction

- There is various agent like DNA damage, oxidative demolition, Telomerase activity and metabolical flow can accelerate aging process.

- Anti-aging medicine goal depend on health span by interference in this pathways.
History

Three hypothesis about aging event

I. Accumulation hypothesis (by Medawar) in 1952

II. Antagonistic pleotropic hypothesis

I. Somatic transmitters (by Kirkwood) in 1996

✓ Anti-aging medicine got current after 2000
Main effective factors in cell senescence (9)

- DNA damage, repair and mutagenesis
- Free radicals & aging
- Age-related disease
- Signal transduction
- Genetic context or gerantogen
- Metabolic flow
- Cancer, Rheumatoid Arthritis, etc.
DNA damage \(^{(3)}\)

**Exogenous sources**
Radiations, chemicals...

**Endogenous sources**
ROS, replication errors, hydrolysis...

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**Accumulation of DNA damage**

- Mutagenic lesions
- Cytotoxic or cytostatic lesions

**DNA repair mechanisms**
Decline of efficiency and accuracy of DNA repair

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**Defects of cellular functions**

**Cell death and senescence**

**AGING**

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Aging and biotechnology relationship
Aging and longevity are controlled by genome

Aging can be suppressed and life span increased

organisms with low half life like nematode caenorabditis elegans are used in gerantogens study
Genetic context, metabolic follow & signal transduction

Insulin/insulin-like growth factor pathway with influence on lifespan

Aging and biotechnology relationship
- Researches discovered with mutation in genes *daf-2* or *age-1* lifespan increase.

- The orthologs gene mammalian respectively
  - *InR* and *PI3K*

*DAF-16* human orthologs is *FOXO*

- **Age-1**
- **Daf-2**

**DAF-16** increases lifespan

- **longevity gene**

✓ Age-1 or daf-2 and their orthologs are suitable target for RNAi.
How free radicals cause aging

[Diagram showing the mechanism of how free radicals cause aging through lipid peroxidation, DNA alterations, RNA alterations, and protein alterations leading to cellular damage, aging, and death.]
Biotechnology role in aging (9)

- Detection, decrease of aging process and increase of health longevity
- Creation of aging process and death in malignant, cancerous and infection cells
- Any one of them have some pharmaceutical target

Aging and biotechnology relationship
Inhibitors and Inducers of Apoptosis proteins

Mitochondrial protein and Membrane potential

Gerantogen activity

Telomerase activity

oxidants

Pharmaceutical targets

Aging and biotechnology relationship
For accomplish the above cases biotechnology Needs some techniques such as:

- RNAi (siRNA & miRNA)
- Drug delivery techniques materials Nano
- Cell therapy, Gene therapy
- Tissue engineering
- Recombinant antibody
- DNA vaccines
RNAi-based therapeutic

- The induction of RNAi relies on small silencing RNA that affect specific messenger RNA (mRNA) degradation.
- siRNA and miRNA have a central function in RNAi technology.
## RNAi-based therapeutic

<table>
<thead>
<tr>
<th>SiRNA target genes</th>
<th>disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-Tat, HIV-Rev, HIV-Vif</td>
<td>Viral</td>
</tr>
<tr>
<td>HPV-E6 &amp; E7, HBV-S1,-S2,-S-X</td>
<td></td>
</tr>
<tr>
<td>CCR5, CXCR4 ,CD4</td>
<td></td>
</tr>
<tr>
<td>P53 mutant, k-Ras, BCR- ABL, MDR1</td>
<td>Cancer</td>
</tr>
<tr>
<td>C-Raf, Bcl2, VEGF, PKC-a, B-catenin</td>
<td></td>
</tr>
</tbody>
</table>
two RNAi delivery strategies to target cancerous and viral Tissues;\(^{(6)}\)

I. Systemic administration

II. Local administration

- Local administration may be promising to overcome systemic delivery disadvantage such as Liver toxicity and stimulation of immune response
RNAi-based therapeutic (6)

- Oncology can benefit the most from this novel therapeutic strategy
- **Lung cancer** is the most common cause of cancer-related death worldwide
Table 1. Small interfering RNA (siRNA)-based therapeutics for cancer treatment in clinical trials.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target Gene</th>
<th>Delivery Methods</th>
<th>Disease</th>
<th>Vehicle</th>
<th>Phase</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALAA-01</td>
<td><em>RRM2</em></td>
<td>Intravenous injection</td>
<td>Solid tumors</td>
<td>Cyclodextrin nanoparticle</td>
<td>I</td>
<td>2008</td>
</tr>
<tr>
<td>TKM 080301</td>
<td><em>PLK1</em></td>
<td>Intravenous injection</td>
<td>Solid tumors with liver involvement</td>
<td>Lipid nanoparticle (LNP)</td>
<td>I/II</td>
<td>2010</td>
</tr>
<tr>
<td>ALN-VSP02</td>
<td><em>KSP/VEGF</em></td>
<td>Intravenous injection</td>
<td>Solid tumors with liver involvement</td>
<td>Lipid nanoparticle (LNP)</td>
<td>I</td>
<td>2009</td>
</tr>
<tr>
<td>Atu027</td>
<td><em>PKN3</em></td>
<td>Intravenous injection</td>
<td>Solid tumors</td>
<td>Lipid nanoparticle (LNP)</td>
<td>I</td>
<td>2009</td>
</tr>
<tr>
<td>siG12D LODER</td>
<td><em>KRAS-G12D</em></td>
<td>EUS biopsy needle</td>
<td>Pancreatic ductal adenocarcinoma</td>
<td>LODER polymer</td>
<td>II</td>
<td>2011</td>
</tr>
<tr>
<td>siRNA-EphA2-DOPC</td>
<td><em>EphA2</em></td>
<td>Intravenous injection</td>
<td>Solid tumors</td>
<td>DOPC</td>
<td>I</td>
<td>2012</td>
</tr>
</tbody>
</table>
Development of small RNA delivery technologies for lung cancer

(a) Target selection for RNAi-based therapeutics
- in vitro assay
- in vivo assay
- database
- clinical samples
- selection candidate microRNAs and target genes for lung cancer treatment from various basic data

(b) Appropriate drug delivery systems
- Each combination of delivery technologies
  - i) Delivery route
  - ii) Delivery carriers
  - iii) Chemical modification
  - iv) RNAi platform
- e.g. intranasal
- e.g. PnkRNA™ and nkRNA®

(c) Animal model study
- Lung cancer mice models
- Collaboration with pharmaceutical companies
- Feasibility study in animal models
- Estimation the effectiveness of RNAi-based therapeutics and delivery strategy

(d) RNAi-based therapeutics for clinical application
- PK/PD test
- Toxicity
- Preclinical study
- (First in human) phase I
- Phase II
- siRNA and microRNA-based therapeutics may be next-generation strategies for lung cancer treatment.
modified siRNA platform (6)

E.g.: PnKRNA(proline naked RNA) and nkRNA(naked RNA)

- With local delivery through inhalation
- More resistant to degradation because of their unique helical structure
- Without inflammatory response in Intrapulmonary delivery
- Lung and ayes, few organs successful
  RNAi could be delivery of naked siRNA
Novel platform SiRNA (8)

A

Human GAPDH PnkRNA

5'-CAUGAGAAAGUAUGACAACAGCC-P-GGCUUGUCUAUCUCUCUGGUUC-P-GAA-3'

Sense Cassette Antisense Cassette

Self-annealing

B

Human GAPDH nkRNA

5'-CAUGAGAAAGUAUGACAACAGGC-CCCACACCCGCCUGUUAGUCAUUCUCUAGGCUGGUUC-3'

Sense Cassette Antisense Cassette

Self-annealing

Aging and biotechnology relationship
P-GP-mediated drug resistance (7)

- P-glicoprotein pomp(p-gp) a member of ABC –transporter family
- Cause drug resistance in some age-related disease
- Such as rheumatoid arthritis(RA) and alzymer
- p-gp an excellent target to control these disease
- TNF-a as a biological target for RA treatment
Relevance of p-Gp in drug resistance (RA)
Apoptosis-targeted therapies

- Apoptosis, programed cell death (PCD) maintains healthy, survival/death balance in metazoan cell.
- Defect PCD cause cancer or autoimmunity. Enhanced PCD cause degenerative disease, immunodeficiency, infertility.
- Inhibitor of apoptotic proteins (IAP) and FLICE-inhibitor protein (cFLIP), central control point of PCD pathways, modulate by carcinogenesis signals.

CFLICE; cellular FADD–like interleukin-1beta converting enzyme.
Table 6.1 The Bcl-2 family of proteins

<table>
<thead>
<tr>
<th></th>
<th>Anti-apoptotic</th>
<th>Pro-apoptotic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mammalian</strong></td>
<td>Bcl-2</td>
<td>Bcl-X_S</td>
</tr>
<tr>
<td></td>
<td>Bcl-X_L</td>
<td>Bax</td>
</tr>
<tr>
<td></td>
<td>Bcl-W</td>
<td>Bad</td>
</tr>
<tr>
<td></td>
<td>Mcl-1</td>
<td>Bak</td>
</tr>
<tr>
<td></td>
<td>A1</td>
<td></td>
</tr>
<tr>
<td><strong>C. elegans</strong></td>
<td>Ced-9</td>
<td></td>
</tr>
<tr>
<td><strong>Viral(^a)</strong></td>
<td>E1B-19K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BHRF1</td>
<td></td>
</tr>
</tbody>
</table>
Apoptosis-targeted therapies

successful nonsurgical cancer cell eradication approached by induction of apoptosis

apoptotic-based therapy is focused on BCL-2 family proteins

Three strategies to overcome cytoprotective effect of BCL-2

- Shutting off gene transcription
- Inducing mRNA degradation by antisense oligonucleotides
- Directly attacking the proteins with small molecular drugs
Some anti apoptotic drug in cancer therapy

<table>
<thead>
<tr>
<th>Targeted molecule</th>
<th>Modulatory function</th>
<th>drug</th>
<th>cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid/Rtinoide transcription factor (RAR, VDR)</td>
<td>Inhibit BCL-2</td>
<td>doxorubicin</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>Interaction with DNA</td>
<td>Lower serum PSA</td>
<td>Traglitazone (As a PPRA agonist)</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>Histone deasetilase</td>
<td>Inhibit BCL-2, BCL-xL</td>
<td>HDAC inhibitors</td>
<td>Some tumors</td>
</tr>
<tr>
<td>BCL-2, BCL-xL</td>
<td>Inhibit BCL-2, MCL</td>
<td>Synthetic BH3 peptide</td>
<td>Follicular lymphoma</td>
</tr>
<tr>
<td>Bak and BaX</td>
<td>Activate Bak and BaX</td>
<td>Synthetic BH3 peptide</td>
<td>-</td>
</tr>
<tr>
<td>CD20</td>
<td>Complement activation</td>
<td>Monoclonal antibody</td>
<td>Certain leukemia</td>
</tr>
</tbody>
</table>
Telomerase-based therapies

- Telomeres are guanine-rich repeated sequences located at the end of chromosomes.
- A number of proteins are located on telomeres which protect telomeres by forming D- and T-loops.
- They function as a biological clock limiting the cell proliferation with every next cell division that accompanied by cell senescence, mitotic crisis, and apoptosis.
Telomere structure

Aging and biotechnology relationship
Telomerase –based therapies

- Most cancer cells (~85%) reveal telomere length maintenance that is responsible for telomeres renewal during cell proliferation.
- Cancer cells utilize telomerase for this purpose.
- The enzyme is a multisubunit rebonucleoprotein contains catalytic subunit and RNA molecule as a template for telomerase reversion transcription (TERT).

Aging and biotechnology relationship
Telomeres and telomerase

Aging and biotechnology relationship
Telomerase-based therapies

There is several strategies for control telomerase in cancer cells

- Antisense technology against telomerase RNA component (TR) and telomerase reves transcriptase
- Ribozyme against TERT
- Modulation of interaction with other proteins involved in the regulation of telomerase and telomeres
Transcriptional mechanism of telomerase regulation

<table>
<thead>
<tr>
<th>Telomerase inducers</th>
<th>Telomerase repressors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin [45]</td>
<td>Dexamethasone (Dex) [60]</td>
</tr>
<tr>
<td>EGFR [46]</td>
<td>EGF [69]</td>
</tr>
<tr>
<td>Survivin* [94]</td>
<td>Gefitinib* [64]</td>
</tr>
<tr>
<td>Sp1 [95]</td>
<td>Genistein (phytoestrogen)* [61]</td>
</tr>
<tr>
<td>Mad1/c-Myc [97, 98]</td>
<td>Upstream stimulatory factor (USF) 1 and 2 [108]</td>
</tr>
<tr>
<td>HBX protein (X protein of HBV) [100]</td>
<td>WT1 (Wilm's tumor 1 suppressor gene product) [110]</td>
</tr>
<tr>
<td>HPV16 E6 [102]</td>
<td>MZF-2 [112, 113]</td>
</tr>
<tr>
<td>ERK/ER81 [28, 44, 103, 104]</td>
<td>p53-Sp1 complex [114]</td>
</tr>
<tr>
<td>HBZ (HTLV1 bZIP factor) [92]</td>
<td>p53/p21/Rb/E2F [95]</td>
</tr>
<tr>
<td>LANA (latency-associated nuclear antigen) [93]</td>
<td>p73 [115]</td>
</tr>
<tr>
<td>Her2/Neu/Ras/Raf [44]</td>
<td>NFX1-91 [116]</td>
</tr>
<tr>
<td>ANP73 [96]</td>
<td>Interferon-γ [117]</td>
</tr>
<tr>
<td>Ets2 [99]</td>
<td>CTCF [118, 119]</td>
</tr>
<tr>
<td>STAT3 [101]</td>
<td>PPARγ (peroxisome proliferator-activated receptor γ) [120]</td>
</tr>
<tr>
<td>Estrogen receptors (ERs) ERα and ERβ [47]</td>
<td>PPAR α (peroxisome proliferator-activated receptor α) [121]</td>
</tr>
<tr>
<td>17β - estradiol (E2) [47, 50]</td>
<td>Menin [106]</td>
</tr>
<tr>
<td>Egr-1(early growth response 1) transcription factor [105]</td>
<td>TR antisense oligonucleotides [77]</td>
</tr>
<tr>
<td>TGFβ [106]</td>
<td>Butein (3,4,2',4'-tetrahydroxychalcone) [64]</td>
</tr>
<tr>
<td>PTEN [107]</td>
<td>Peceotenoxin-2 (PTX2) [65]</td>
</tr>
<tr>
<td>IP6* [109]</td>
<td>Imetelstat sodium (GRN163L) [68]</td>
</tr>
<tr>
<td>Imatinib mesylate* [111]</td>
<td>ATRA (all-trans retinoic acid) [71]</td>
</tr>
<tr>
<td>Indole-3-carbinol (I3C)[51]</td>
<td>Melatonin [63]</td>
</tr>
<tr>
<td>BRCA1 gene [52, 53]</td>
<td>Butein (3,4,2',4'-tetrahydroxychalcone) [64]</td>
</tr>
<tr>
<td>Nmi (N-myc and c-myc interacting protein) [54]</td>
<td>Butein (3,4,2',4'-tetrahydroxychalcone) [64]</td>
</tr>
<tr>
<td>Rad50 [55]</td>
<td>Pectenotoxin-2 (PTX2) [65]</td>
</tr>
<tr>
<td>Raloxifene [62]</td>
<td>Imetelstat sodium (GRN163L) [68]</td>
</tr>
<tr>
<td>Melatonin [63]</td>
<td>ATRA (all-trans retinoic acid) [71]</td>
</tr>
</tbody>
</table>

Aging and biotechnology relationship
Telomerase-based therapies

Natural-derived compounds with antitumor activity in the context of telomerase regulation in breast cancer

- **butein** (3,4,2’,4’-tetrahydroxy chalcone) it cause downregulation by suppression of TERT gene expression in a C-myc depending manner.

- **pectenotxin-2** dependent attenuation of TERT expression was mediated through suppression of C-myc and sp1-binding on the regulatory region of TERT.

- C-myc have role in TERT gene expression induction.
Telomerase-based therapies (4)

silencing TERT and HER-2 (human epithelial grow factor-2) by SiRNA technology increased radiosensitivity and down regulation TERT/telomerase
Anticancer DNA vaccine based on human telomerase reverse transcriptase generates a strong and specific T cell immune response.

Thalmensi J1, Pliquet E2, Liard C1, Escande M1, Bestetti T1, Julitte M1, Kostrzak A1, Pailhes-Jimenez AS1, Bourges E1, Loustau M1, Caumartin J1, Lachgar A1, Huet T1, Wain-Hobson S2, Langlade-Demoyen P2.

Abstract

Human telomerase reverse transcriptase (hTERT) is overexpressed in more than 85% of human cancers regardless of their cellular origin. As immunological tolerance to hTERT can be overcome not only spontaneously but also by vaccination, it represents a relevant universal tumor associated antigen (TAA). Indeed, hTERT specific cytotoxic T lymphocyte (CTL) precursors are present within the peripheral T-cell repertoire. Consequently, hTERT vaccine represents an attractive candidate for antitumor immunotherapy. Here, an optimized DNA plasmid encoding an activated form of hTERT, named INVAC-1, was designed in order to trigger cellular immunity against tumors. Intradermal injection of INVAC-1 followed by electrogene transfer (EGT) in a variety of mouse models elicited broad hTERT specific cellular immune responses including high CD4+ Th1 effector and memory CD8+ T-cells. Furthermore, therapeutic INVAC-1 immunization in a HLA-A2 spontaneous and aggressive mouse sarcoma model slows tumor growth and increases survival rate of 50% of tumor-bearing mice. These results emphasize that INVAC-1 based immunotherapy represents a relevant cancer vaccine candidate.

KEYWORDS: Cancer; DNA vaccines; electrogene transfer; electroporation; hTERT; immunotherapy
Recent natural anti-aging compounds

<table>
<thead>
<tr>
<th>Compound name</th>
<th>source</th>
<th>application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape-seed extract</td>
<td>Fruit polyphenols&amp; flavonoids</td>
<td>Skin treatment</td>
</tr>
<tr>
<td>Jojoba protein</td>
<td>Jojoba plant</td>
<td>Moisturizer &amp; anti-wrinkle</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>Plant phenolic compounds</td>
<td>Anti-oxidant &amp; anti-inflammatory</td>
</tr>
<tr>
<td>Berries</td>
<td>-</td>
<td>Cancer &amp; improve brain function</td>
</tr>
<tr>
<td>Extra virgin olive oil</td>
<td>olive</td>
<td>Reduce aging, cancer &amp; cardiovascular diseases</td>
</tr>
<tr>
<td>lycopene</td>
<td>tomato</td>
<td>Reduce cancer &amp; aging risk</td>
</tr>
<tr>
<td>Spirulalia</td>
<td>plankton</td>
<td>Immune-stimulator &amp; moisturizer</td>
</tr>
<tr>
<td>Dark chocolates</td>
<td>Cocoa bean</td>
<td>Reduce skin inflammatory</td>
</tr>
<tr>
<td>Kojic acid dipalmitate</td>
<td>Japanese mushroom</td>
<td>Invigorating &amp; skin lightening</td>
</tr>
</tbody>
</table>
Nano biotechnology effect in increasing longevity

- advance in joint displacement
- noninvasive & invasive diagnosis
to fast disease follow up
- co-diagnostic devices
- cancer wise treatment

- artificial organs
- in vivo sensors
- local drug delivery
- nerves stimulation
- heart materials & cordial disease treatment

- development of cell-material interaction
- tissue scaffold engineering
- Genetical treatment of cell senescence
- treatment by stem cells

- local drug delivery
- gene therapy expansion
- foppish structures
- fast diagnostic approaches
Conclusion

- Although aging is gradual and spontaneous changes in structure and function of living creature with time passing.

- Some disease such as cardiovascular, cancer and accidents are the main cause of death and early aging.

- Up to now the disease treatment were based on chemotherapy drugs, different rays and surgery.
Nowadays with use of medical biotechnology and related field to be able to Take a little step in order to have a health senescence cycle by;

- Early diagnosis of disease, follow up treatment and recovery
- Efficient delivery of drug to target point, make organ and different biochemical sensors
- Genetical drugs based on RNA and DNA with attention to patient genetical features
References

References


پاری. پیری سلولی و بیوتکنولوژی. مجله تازه های بیوتکنولوژی سلولی-مولکولی. 2011;1(4):7-9

Aging and biotechnology relationship
Thank for your time