Einfluss pflanzlicher Substanzen auf Telomere und Epigenetik

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Telomere und Telomerase
Telomere Structure und Function (1)

Consist of repeat sequences and associated proteins

Cap the ends of chromosomes - protection against end-end-fusions, recombinations, degradation

Essential for chromosomal integrity

Nabetani A, and Ishikawa F J Biochem 2011;149:5-14
Telomere Structure und Function (2)

Acting as buffers to prevent loss of genetic information.

What We Lose With Age

As cells divide over time...

Telomeres shorten, and eventually cell division stops.
Telomerase Structure und Function (1)

Composed of RNA subunits (hTR) Reverse transcriptase catalytic subunit (hTERT)

Telomerase elongates telomeres which are shortened after each replication cycle

Additional functions:
- Regulation of genexpression via NF-kB pathway or Wnt/ß-catenin Weg
- DNA damage repair
- Promotes cell survival under oxidative stress or endoplasmic reticulum stress conditions
- Protects developing neurons from DNA-damage induced cell death
- Regulates mitochondrial function and cell metabolism

Zhou et al. 2013, Fu et al. 2000, Handeler et al. 2009,
Senescence

Telomere shortening
50-200 bp per replication

Telomere crisis: Telomere critical short – DNA damage response

P53 and p16/pRB pathway induce apoptosis or senescence

Cancer Prevention in young
Organismal aging in the elderly

Defect of pathways
Cells continue to divide, chromosomes get instable

Second Crisis
Telomerase activation leads to immortalisation

In the young: cancer prevention
In the elderly: organismal aging

Malignant transformation

Frontiers in Bioscience 14, 4044-4057, January 1, 2009
Therapy? (1)

• High telomerase activity observed in most invasive metastatic tumours – telomerase inhibition as target?
• natural compounds can inhibit telomerase activity and induce apoptosis

Cosan and Soyocak 2012
• Resveratrol treatment in MCF-7 breast cancer cells down-regulated the telomerase activity of target cells and the nuclear levels of hTERT
• Resveratrol showed direct antiproliferative and pro-apoptotic effects

• Resveratrol dose-dependently inhibited the onset of EPC senescence in culture and increased telomerase activity and the expression of the catalytic subunit, hTERT
• Resveratrol delayed EPCs senescence in vitro, which may be dependent on telomerase activation!
EGCG dose-dependently inhibited telomerase activity (40-55%) and inhibited the mRNA expression (40-55%) of hTERT leading to the suppression of cell viability and induction of apoptosis.

Epigallocatechin gallate induces telomere fragmentation in HeLa and 293 but not in MRC-5 cells. EGCG causes telomere fragmentation and therefor an apoptosis-inducing effect in HeLa, but not in normal cells (MRC-5 cells) (it seems to work specifically in cancer cells)
Telomere length in Caco-2 cells without treatment

Picture 1: Time kinetics of telomere length in Caco-2 human colon adenocarcinoma cells measured by qPCR (method by O’Callaghan and Fenech 2011)
Telomere length in Caco-2 Cells after 120h treatment with 200µM EGCG and 20 µM Equol

Picture 2: Telomere length in Caco-2 human colon adenocarcinoma cells measured by qPCR (O’Callaghan and Fenech 2011)
In TRF-treated senescent HDFs, elongated telomere length and restoration of telomerase activity were observed.

preventing HDFs cellular ageing by restoring telomere length and telomerase activity, reducing damaged DNA, and reversing cell cycle arrest associated with senescence.
Therapy? (2)

Telomere dysfunction by
- Inflammatory processes that lead to increased turnover of cells contribute to attrition of telomeric repeats by increasing cell divisions
- Oxidative stress

Influence of nutrients on telomere length via
- Influencing telomerase activity
- Mechanisms that reflect their role in cellular functions including DNA repair and chromosome maintenance, DNA methylation, inflammation, oxidative stress and activity of the enzyme telomerase
- Antiinflammatory and antioxidant properties
- Influencing regulation of telomere length: e.g., folate via its role in epigenetic status of DNA and histones, and nicotinamide through its role as a substrate for posttranslational modification of telomere associated proteins.

*Telomere length is epigenetically regulated by DNA and histone methylation*
Genomic instability and Cancer

Cancer has been described as a disease of genomic instability.

Cancer initiation and progression driven by changes in expression of multiple genes via genetic and epigenetic alterations.

Half of gene defects that occur in cancer through epigenetic alterations.

Activation of oncogenes and prometastatic genes or silencing tumour suppressor genes and to genome rearrangements and instability
Dietary Epigenetics in Cancer and Ageing

Three important epigenetic mechanisms in mammals:

- DNA methylation
- Histone modification
- non coding RNAs

Feinberg and Tycko, 2004
DNA methylation

Three major methyltransferases:
- DNMT1
- DNMT3A
- DNMT3B

*In general, the more methylated a gene regulatory region becomes, the less transcription will occur from the promoter.*

Feinberg and Tycko, 2004
Histone modifications

- histone acetylation, methylation, phosphorylation, ubiquitination, biotinylation, sumoylation, ADP-ribosylation
- Large number of enzymes, most interesting regarding aging and cancer: HAT, HDACs

*In general the more acetylated the histone amino tails become, the more likely it is that the gene promoter region that contains those histones will increased transcriptional activity*

Feinberg and Tycko, 2004
Clayton et al. 2006
Non coding RNA

• Single stranded non coding RNAs
• 21-23 nucleotides in length
• suppress gene expression by altering the stability of gene transcripts and also by targeting the transcripts for degradation
• miRNA may also lead to an increase in gene transcription

Feinberg and Tycko, 2004
(Mathers et al. 2010)
Cancer epigenetics

Half of gene defects that occur in cancer through epigenetic alterations:
• General hypomethylations in cancer cells
• Gene specific hypermethylation of tumor suppressors
• Increased activity of HDACs common in cancer cells
• Alteration in miRNAs in cancer cells

Growing interest in collective interactions of epigenetic processes in the control of gene regulation aberrations in cancer.
Epigenetics diet in cancer prevention and treatment

Epigenetics diet that impacts the epigenome and that may be used in conjunction with other cancer prevention and chemotherapeutic strategies.

Many natural dietary agents which consist of bioactive compounds have been shown to be effective in cancer prevention and treatment and these nutraceuticals often mediate favorable epigenetic changes.
Examples of dietary ingredients with epigenetic and chromatin remodeling properties

- Sulforanes from Brassica – HDAC inhibitors
- EGCG from green tea – DNA demethylation
- Genistein from soy – DNA methylation/demethylation
- Resveratrol from red grapes – affects NAD⁺- dependent histone deacetylases (i.e., SIRT1) that deacetylates histones and regulatory proteins like PGC-1α
- Lunasin from soy – chromatin binding peptide and inhibitor of histone acetylation
<table>
<thead>
<tr>
<th>Natural compound</th>
<th>Natural sources</th>
<th>Pharmacological effects</th>
<th>Epigenetic mechanisms of action</th>
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</table>
| Folate, cobalamin, riboflavin, pyridoxine, methionine | Folate and riboflavin: spinach, asparagus, beans, peas, lentils, sunflower seeds, almonds  
Cobalamin: fish, shellfish, poultry, milk, eggs  
Pyridoxine and methionine: grains, nuts, dragon fruit, sesame seeds | Anti-cancer, anti-proliferative, chemoprevention of malignant transformation | Regulation of one-carbon metabolism, SAM/SAH ratio, DNMT and MBD expression; regulation of miRNAs (tumour suppressor miR-122, miR-34a, miR-127, and oncogenic miR-21, miR-222) |
<p>| Retinoic acid             | Vietnamese gac, crude palm oil, yellow and orange fruits (mango, papaya), orange root vegetables (carrots), spinach, sweet potatoes | Anti-cancer, anti-proliferative, differentiating, pro-apoptotic | Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1, PTEN and ERs; regulation of miRNAs targeting DNMTs; regulation of tumour suppressor miRNAs (miR-15, miR-16, let-7a, let-7c, miR-34a, miR-342) and oncogenic miRNAs (miR-10a); GNMT regulation; histone acetylation |
| Vitamin D3                | Sun exposure, fish, fish liver oils                                             | Anti-cancer, anti-proliferative, differentiating, pro-apoptotic | Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1, PTEN and ERs; regulation of histone acetylation; regulation of oncogenic miRNAs (miR-181a, miR-181b) |
| Resveratrol               | Roots of hellebore, grapes, mulberries, apricots, pineapples, peanuts           | Anti-cancer, antioxidant, anti-proliferative, anti-angiogenesis, anti-inflammatory, pro-apoptotic, cardioprotective | Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1 and PTEN; activation of deacetylase SIRT1 and p300 HAT; down-regulation of UHRF1; regulation of miRNAs |</p>
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<tr>
<th>Genistein and daidzein</th>
<th>Soybeans, lupin, kudzu, psoralea, fava beans, coffee</th>
<th>Anti-cancer, antioxidant, antihelminthic, anti-metastatic, cancer protective</th>
<th>Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1 and PTEN; increase in HAT activity; regulation of miRNAs (tumour suppressor miR-1296, miR-16, and oncogenic miR-27a)</th>
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<tbody>
<tr>
<td>EGCG</td>
<td>Green tea</td>
<td>Anti-cancer, antioxidant, anti-proliferative, anti-angiogenesis, anti-inflammatory, pro-apoptotic, cancer protective</td>
<td>Regulation of SAM/SAH ratio by COMT-mediated reactions; direct inhibition of DNMTs by binding to catalytic domain of the enzyme; regulation of tumour suppressor miRNAs (miR-16)</td>
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<td>Curcumin</td>
<td>Spice turmeric</td>
<td>Anti-cancer, antioxidant, protects against heart failure</td>
<td>Direct inhibition of DNMTs by binding to catalytic domain of the enzyme; inhibition of HDACs and p300 HAT; regulation of miRNAs (tumour suppressor miR-22, miR-15a, miR-16, and oncogenic miR-21, miR-199a)</td>
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Flavonoids inhibit DNA methyltransferases (DNMTs)

Apigenin

DNMT inhibitor (Fang M. et al 2007)

Meeran, et al. 2010
Flavonoids act as HDAC inhibitors or HATs (histone deacetylase inhibitors or histone acetyl transferases)

Genistein

HDAC inhibitor and HAT activator
(Majid et al. 2008)
Meeran, et al. 2010
Flavonoids act on mRNAs

Reuter S., et al. 2011
EGCG

Many studies have shown a positive correlation between green tea (EGCG) consumption and the inhibition of numerous cancers (Kim et al. 2010; Chen et al. 2011; Shanmugan et al. 2011).

Can inhibit DNMT activity by directly interacting with the DNMTs (Fang et al. 2003) - effectively lead to the reversal of tumor suppressor epigenetic silencing of cancer cells and induce apoptosis of these cells.

Inhibition of DNMTs by EGCG can lead to the suppression of telomerase in cancer cells by down-regulating hTERT (Mittal et al. 2004; Berletch et al. 2008).

hTERT activity in cancer cells is associated with increased DNA methylation of its gene regulatory region due to repressors binding to its promoter (Berletch et al. 2008).
Ageing

Aging is associated with general genomic hypomethylation and regional or gene-specific hypermethylation

(Mays-Hoopes 1989; Issa 1999)
Ageing

• epigenetic diet components may also have advantageous effects on longevity through their cancer preventive properties

Consumption of components of the epigenetics diet over a period of time may lead to a decrease in age-associated diseases such as cancer and cardiovascular disease (Mathers 2006).
Conclusio

• Various nutrients influence telomere length via mechanisms that reflect their role in cellular functions including DNA repair
• Anti-inflammatory and antioxidant nutrients can reduce erosion of telomeres
• have anticancer epigenetic effects and are also efficacious for preventing or treating the epigenetic aberrations of other age-associated diseases besides cancer
Prospectives

In vivo mice: Mausversuch
• Analyse
• Telomerlänge (qPCR), Telomerase
• Methylierung TERT (Telomerase Gen)
• Methylierung Glucocorticoidrezeptor, Estrogenrezeptor
• Entzündungsparameter
• Mikrobiota
• DNA Schäden

Humanstudie mit Timeblock, Complex Telomer
• 8 Monate
• Analyse: Schleimhaut, Blutropfen, Stuhl
• Telomerlänge (qPCR), Telomerase
• Methylierung TERT (Telomerase Gen)
• Methylierung Glucocorticoidrezeptor, Estrogenrezeptor
• Entzündungsparameter
• Mikrobiota
• DNA Schäden
Thank you...

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