Oxidative Damage? Not a Problem! The Characterization of Humanin-like Mitochondrial Peptide in Anoxia Tolerant Freshwater Turtles

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Abstract

Mitochondria was long thought to be an "end function" organelle that regulated the metabolic flux and apoptosis in the cell. However, with the discovery of humanin (HN/MTRNR2), the first mitochondrial peptide in the early 2000s, the cytoprotective and pro-survival applications of MDPs have taken the forefront of therapeutic and diagnostic research. However, the regulation of humanin-like MDPs in natural model systems that can tolerate lethal environmental and cytotoxic insults remains to be investigated. Red-eared sliders are champion anaerobes that can withstand three continuous months of anoxia followed by rapid bouts of oxygen reperfusion without incurring cellular damage. Freshwater turtles employ extensive physiological and biochemical strategies to combat anoxia, with metabolic rate depression and a global enhancement of antioxidant and cytoprotective pathways being the two most important contributors. The main aim of this study was to uncover and characterize the humanin-homologue in freshwater turtles as well as investigate the differential regulation of humanin in response to short and long-term oxygen deprivation. In this study we have used de novo and homology-based protein modelling to elucidate the putative structure of humanin in red-eared sliders as well as an ELISA and western immunoblotting to confirm the protein abundance in the turtle brain and six peripheral tissues during control, 5 h, and 20 h anoxia (n=4/group). We found that a humanin-homologue (TSE-humanin) is present in red-eared sliders and it may play a crucial cytoprotective role against oxidative damage.

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A

```
#
#
#  Percent Identity  Matrix - created by Clustal2.1
#
#

     1: H.sapien_Humanin           100.00   74.67
     2: T.s.elegans_mitochondrion   74.67  100.00
```

B

```
H.sapien_Humanin                ------------------------------------------------------------    0
T.s.elegans_16SrRNA             ------------------------------------------------TGTCTTCTAAATAAA    15
T.s.elegans_mitochondrion       TAAACGGCCGCGGTATCCTAACCGTGCAAAGGTAGCGTAATCACTTGTCTTCTAAATAAA    2100

                                                                                  TSE-humanin
H.sapien_Humanin                ----------ATGGCTCCACGAGGGTTCAGCTGTCTCTTACTTTTAACCAGTGAAATTG    49
T.s.elegans_16SrRNA             GACTAGAATGAATGGCCAAACGAGGTTCTACCTGTCTCTTACAGATAATCAGTGAAATTG    75
T.s.elegans_mitochondrion       GACTAGAATGAATGGCCAAACGAGGTTCTACCTGTCTCTTACAGATAATCAGTGAAATTG    2160
                                     *****   ****** *  * ***********   *** ***********

H.sapien_Humanin                ACCTGCCCGTGAAGAGGCGGGCATAA----------------------------------    75
T.s.elegans_16SrRNA             GTCTCCCCGTGCAAAAGCGAGGATAATCCTATAAGACGAGAAGACCCTGTGGAACTTTAA    135
T.s.elegans_mitochondrion       GTCTCCCCGTGCAAAAGCGAGGATAATCCTATAAGACGAGAAGACCCTGTGGAACTTTAA    2220
                                  ** ****** * * *** * ****

H.sapien_Humanin                ------------------------------------------------------------    75
T.s.elegans_16SrRNA             ACACAAATCAACTACCATCAAACACCAAACTAAGGATTTATGACCAACTAGTATATGATC    195
T.s.elegans_mitochondrion       ACACAAATCAACTACCATCAAACACCAAACTAAGGATTTATGACCAACTAGTATATGATC    2280

H.sapien_Humanin                ------------------------------------------------------------    75
T.s.elegans_16SrRNA             CACGTTTTCGGTTGGGGCGACCTCGGAGTAAAGCACAACCTCCGAAAAAAGAGCATACTT    255
T.s.elegans_mitochondrion       CACGTTTTCGGTTGGGGCGACCTCGGAGTAAAGCACAACCTCCGAAAAAAGAGCATACTT    2340

H.sapien_Humanin                ------------------------------------------------------------    75
T.s.elegans_16SrRNA             CTCTAAACCTAGACTTACAACTCAAAGTGCTAACAGCAAAAAGATCCAATATATTTGATC    315
T.s.elegans_mitochondrion       CTCTAAACCTAGACTTACAACTCAAAGTGCTAACAGCAAAAAGATCCAATATATTTGATC    2400

H.sapien_Humanin                ------------------------------------------------------------    75
T.s.elegans_16SrRNA             AACGAACTAAGCTACCCCAGGGATAACAGCGCAATCCCATCCTAGAGCCCTTATCGACGA    375
T.s.elegans_mitochondrion       AACGAACTAAGCTACCCCAGGGATAACAGCGCAATCCCATCCTAGAGCCCTTATCGACGA    2460

H.sapien_Humanin                ------------------------------------------------------------    75
T.s.elegans_16SrRNA             TGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTGATGGTGAAACCGCTATCAAGGG    435
T.s.elegans_mitochondrion       TGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTGATGGTGAAACCGCTATCAAGGG    2520

H.sapien_Humanin                ------------------------------------------------------------    75
T.s.elegans_16SrRNA             TTCGTTTGTTCAACGATTAATAGT------------------------------------    459
T.s.elegans_mitochondrion       TTCGTTTGTTCAACGATTAATAGTCCTACGTGATCTGAGTTCAGACCGGAGCAATCCAGG    2580

H.sapien_Humanin                ------------------------------------------------------------    75
T.s.elegans_16SrRNA             ------------------------------------------------------------    459
T.s.elegans_mitochondrion       TCGGTTTCTATCTATATGAATCTTGAGTCTTTTCTAGTACGAAAGGACCGAAAAGACAGG    2640
```

A

```
T.S.elegans_M     1 MAKRGSTCLLQMISEIGLPVQKRG
T.S.elegans_R     1 MAKRGSTCLLQIISEIGLPVQKRG
consensus         1 ************.************
```

95.83 % Sequence Identity

B

Predicted Humanin Sequence (Mitochondrial)

```
T.S.elegans_M     1 MAKRGSTCLLQMISEIGLPVQKRG
H.sapien_M        1 MAPRGFSCLLLLTSEIDLPVKRA-
consensus         1 **.**..***...***.***....
```

56.52 % Sequence Identity

C

Predicted Humanin Sequence (Nuclear)

```
T.S.elegans_R     1 MAKRGSTCLLQIISEIGLPVQKRG
H.sapien_R        1 MAPRGFSCLLLLTSEIDLPVKRRA
consensus         1 **.**..***...***.***..*.
```

58.33 % Sequence Identity

D

Mitochondrial-coded Humanin

```
T.s.elegans       1 MAKRGSTCLLQMISEIGLPVQKRG-
C.p.belli         1 MAKRGSTCLLQMISEIDLPVQKRG-
H.sapien          1 MAPRGFSCLLLTSEIDLPVK-RA-
M.fascicularis    1 MAPRGFNCLLLTSEIDLSVK-RT-
T.francoisi       1 MAPRGFNCLLLTSEIDLPVK-RAW
R.norvegicus      1 MAKRGFNCLLLSISEIDLPVK-LES
I.tridecemlinea   1 MAWRGFNCLLLISEIDLPVK-REF
C.lateralis       1 MAQRGFNCLLLISEIDLPVK-REF
S.vulgaris        1 MAPRGLNCLLLISELAFPW-GGD-
T.sibiricus       1 MAWRGFNCLLLPISEIDLPVK-REY
T.swinhoei        1 MAKRGFICLLLSISEIDLPVK-RE-
M.lucifugus       1 MAQRGFYCLLLLISEIDLPVK-RE-
M.davidii         1 MALRGFNCLLLSISEIDLPVK-RE-
C.familiaris      1 MATRGFNCLLLPISEIDLPVK-REY
B.tauris          1 MAARGFYCLLLPISEIDLPVK-REC
consensus         1 ** **. ***. .**.........
```

Nuclear-coded Humanin

```
T.s.elegans       1 MAKRGSTCLLQIISEIGLPVQKRG-
C.p.belli         1 MAKRGSTCLLQIISEIDLPVQKRG-
H.sapien          1 MAPRGFSCLLLTSEIDLPVKRRA-
M.fascicularis    1 MAPRGFNCLLLTSEIDLSVKRRT-
T.francoisi       1 MAPRGFNCLLLTSEIDLPVKRRA-
R.norvegicus      1 MAKRGFNCLLLSISEIDLPVKRLES
I.tridecemlinea   1 MA-RGFNCLLLISEIDLPVKRREF
C.lateralis       1 MAQRGFNCLLLISEIDLPVKRREF
S.vulgaris        1 MAPRGLNCLLLISELAFP-RGGD-
T.sibiricus       1 MA-RGFNCLLLPISEIDLPVKRREY
T.swinhoei        1 MAKRGFICLLLSISEIDLPVKRRE-
M.lucifugus       1 MAQRGFYCLLLLISEIDLPVKRRE-
M.davidii         1 MALRGFNCLLLSISEIDLPVKRRE-
C.familiaris      1 MATRGFNCLLLPISEIDLPVKRREY
B.tauris          1 MAARGFYCLLLPISEIDLPVKRREC
consensus         1 ** **. ***. .**.........
```