The Ketogenic Diet Induces Epigenetic Changes that Play Key Roles in Tumour Development

Jessica Preston, MRes Clinical Research: Human Nutrition
Supervisor: Dr. Nelofer Syed
John Fulcher Molecular Neuro-Oncology Laboratory
Glioblastoma Multiforme

- Glioblastoma multiforme (GBM) is a highly aggressive form of brain cancer

Survival Rate of GBM based on Status of Tumour Crossing the Midline

- Current Treatment includes:
  - Complete surgical resection → chemotherapy with temozolomide + radiotherapy


Failure of Current Therapeutics: Tumour Heterogeneity

Temozolomide resistance

Metastatic phenotype

Necrotic core

Hypoxic region

Tumour relapse

Normoxic region

Resistence to radio-therapy

The Warburg Effect is Universal

Metabolic Therapy

Therapeutic techniques aimed to target the unique metabolism of tumour tissue.

Glucose Availability

Tumour Growth


The Ketogenic Diet as a Form of Metabolic Therapy

- Macronutrient breakdown of a ketogenic diet
  - 75% Fat
  - 25% Protein
  - Few carbohydrates
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Blood Glucose and $\beta$-Hydroxybutyrate Concentrations


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Ketone Bodies

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![Blood Ketones and Glucose Concentrations](image)

Blood Glucose and β-Hydroxybutyrate Concentrations

- Blood Ketones
- Blood Glucose
The Ketogenic Diet as a Form of Metabolic Therapy

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Blood Glucose and β-Hydroxybutyrate Concentrations


Ketogenic Diet Increases Life Expectancy of Glioma in vivo

Survival Curve GL261 Mouse Glioma

Collaborators:
Dr. Adrienne Scheck
Associate Professor
Barrow Neurological Institute

The Ketogenic Diet Potentiates the Effects of Radiation

Survival Curve GL261 Mouse Glioma Radiation Therapy and Ketogenic Diet

Tumour Bioluminescence

Radiation Only

Radiation + KetoCal

Day 3

Day 18

Day 36

β-Hydroxybutyrate (BHB) Treatment Reduces Cell Proliferation in vitro

Established Cell Lines

Primary Cell Lines

Ella Qingyu Zeng
Julianna Stylianou
BHB Enhances the Effectiveness of Radiation and Temozolomide *in vitro*

**Radiation Day 6 and BHB**

- Gy0
- Gy2
- Gy4

**Temozolomide Day 6 and BHB**

- Untreated
- Day 6 10mM BHB
- Day 18 10mM BHB

Sophie Glover
Julianna Stylianou
What is the Mechanism of Action of the Ketogenic Diet?

- Epigenetics changes
- microRNA expression
- chromatin modifying enzymes

Global microRNA Microarray: Animal Model

Differentially Expressed miRs in Standard vs. Ketogenic Diet

- mmu-miR-30a-5p
- mmu-miR-139-5p
- mmu-miR-149-5p
- mmu-let-7e-5p
- mmu-miR-541-5p
- mmu-miR-138-5p (138-2)
- mmu-let-7f-5p
- mmu-miR-346-5p
- mmu-miR-128-3p
- mmu-miR-204-5p
- mmu-miR-92b-3p
- mmu-miR-138-5p (138-1)

Differential miR Expression SD vs. KD Large Fold Change (FC>8)

- mmu-miR-30a-5p
- mmu-miR-139-5p
- mmu-miR-149-5p
- mmu-let-7e-5p
- mmu-miR-541-5p
- mmu-miR-138-5p (138-2)
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- mmu-miR-128-3p
- mmu-miR-204-5p
- mmu-miR-92b-3p
- mmu-miR-138-5p (138-1)
microRNA Changes in KD Target Anti-Cancer Pathways

WikiPathways 2016 Analysis of Differentially Regulated microRNAs

- Parkinsons Disease Pathway_Homo sapiens_WP2371
- Alzheimers Disease_Homo sapiens_WP2059
- miRs in Muscle Cell Differentiation_Homo sapiens_WP2012
- Cell Differentiation - Index_Homo sapiens_WP2029
- miRNAs involved in DNA damage response_Homo sapiens_WP1545
- miRNA Regulation of DNA Damage Response_Homo sapiens_WP1530
- Hematopoietic Stem Cell Differentiation_Homo sapiens_WP2849
- MicroRNAs in cardiomyocyte hypertrophy_Homo sapiens_WP1544
- SRF and miRs in Smooth Muscle Differentiation and Proliferation_Homo sapiens_WP1991
- Metastatic brain tumor_Homo sapiens_WP2249

Specific Gene Targets of Differentially Regulated miRs

- miR-300: p53, mTOR
- miR-99a: Let-7a-1, cMYC
- miR-92b: WNT Signalling
- miR-503: VEGF
- miR-128-1: BMI1

miR Expression in KD Treated vs. Standard Tumour Tissue

- 23% changes in KD mirror healthy tissue
- 55% changes in KD mirror neoplastic tissue
- 22% unknown changes in neoplastic tissue
KD Upregulates miR-138 *in vivo* and *in vitro*
KD Induced Upregulation of miR-138 Targets H2AX

miR-138-1 \[\rightarrow\] H2AX

H2AX

<table>
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<tr>
<th>Normalised Expression</th>
<th>D4</th>
<th>D8</th>
<th>D18</th>
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<tr>
<td>Untreated</td>
<td>1.0</td>
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<td>1.0</td>
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<tr>
<td>10mM BHB</td>
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</table>

Sophie Glover
Julianna Stylianou

KD Influence Expression of Chromatin Modifying Enzyme Expression in vivo

Enzyme Expression Ketogenic vs. Standard Diet

Down Regulated Genes in KD vs. SD

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<th>Gene Symbol</th>
<th>Fold Regulation</th>
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<td>Aurkb</td>
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Up Regulated Genes in KD vs. SD

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Qiagen Array
KD Induces Up Regulation of PRMT8

Simandi Z. et al. PRMT1 and PRMT8 Regulate Retinoic Acid Dependent Neuronal Differentiation with Implications to Neuropathology Stem Cells (2014).
KD Down-regulates PRMT8 Target Genes: CXCR4 and DHFR

**DHFR Expression in Mouse Model**

- **DHFR in vitro**
  - **DHFR**
    - Dihydrofolinic acid donor
    - Thymidylate synthesis
    - Enzyme used for DNA synthesis and repair

- **CXCR4 in vitro**
  - **CXCR4**
    - Chemokine receptor for CXCL12
    - Maintenance of GBM perivascular niche
    - Tied to increased migration
Retinoic Acid (RA) and BHB Reduce Cell Proliferation \textit{in vitro}

Protein Arginine Methyltransferase 8

In Conclusion

- BHB treatment decreases cell proliferation alone and in combination with:
  - Radiation therapy
  - Temozolomide
  - Retinoic Acid

- Changes in epigenetic expression with KD implementation including: microRNAs and chromatin modifying enzymes

- microRNA-138 upregulation in the KD may lead to down regulation of H2AX

- PRMT8 upregulation in KD may lead to down regulation of
  - DHFR
  - CXCR4
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Collaborators
Dr. Adrianne Scheck
Barrow Neurological Institute
Phoenix Arizona

Clinical Team
Mr. Kevin O’Neal
Consultant Neurosurgeon
Mr. Matt Williams
Consultant Oncologist
Mr. Babar Vaquus