Post-Genomic and Post-Transcriptional Mechanisms in Breast Cancer

John Le Quesne
Agenda

- Hello
- The central dogma
- Introduction to translational control
- Dysregulated mRNA translation and breast cancer
  - Phenotype
  - Translation profiling
  - Relationship with survival
- Implications for tumour biology and therapy
The Central Dogma

DNA \xrightarrow{\text{Transcription, processing}} \text{mRNA} \xrightarrow{\text{Translation}} \text{Protein}

Data Storage \rightarrow \text{Information} \rightarrow \text{Structure and Function}
Gene Expression Profiling

- Actually ‘total mRNA abundance profiling’
  - Transcriptional program plus mRNA stability
  - Poor measure of gene expression
Gene Expression Profiling

Deep proteome and transcriptome mapping of a human cancer cell line

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Why doesn’t mRNA abundance predict protein expression?

Postranscriptional control

- Regulated translation
- mRNA is no guarantee of protein

So measure translation instead?

Translating mRNAs strongly correlate to proteins in a multivariate manner and their translation ratios are phenotype specific

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Translational Regulation

- An additional layer of genetic regulation
- Therapeutic potential
- Several analogies with transcription
  - Initiation is the limiting step
  - Cis- and trans-acting factors
  - Global and message-specific translation initiation factors
  - Several such factors are established oncogenes/tumour suppressors
Anatomy of a mRNA

Initiation

Elongation

Termination

5’UTR

80S

40S

AUG

ORF

UAA

3’UTR

AAAAAAA
5’UTRs

- Variable
- Length/structure are inhibitory to translation
- G/C content related to structure
eg \textit{c-myc} 5' UTR

\begin{verbatim}
1
GGGCTTTATCTAATCGCTGTAATCCAGCGAGGGAGGAGCGAGGCGAGGCGGCGGGGC
GGC
61
CGGCTAGGGTGGAAGAGCCGGCAGCGAGGTGGGCTGCTGCGGGCGTCCTGGGAAG
GGAGA
121
TCCGGAGCGAATAGGGGGCTTCGCCTCTGGCCCAGCCCTCCCGCTGATCCCCCAGC
CAGC
181
GGGTGGCAACCCTTTGCCGCATCCACGAAATGGCCCAGCAGCGGGGGCACTTTGC
241
\end{verbatim}
Cap-dependent Initiation

eIF4A Helicase activity

eIF4A

eIF4G

eIF4E

40S Ribosomal Subunit

40S subunit scanning and further unwinding

AUG

60S Ribosomal subunit

Translation elongation & polypeptide synthesis
5’UTR structure is necessary for miRNA activity

Translational Repression and eIF4A2 Activity Are Critical for MicroRNA-Mediated Gene Regulation

H. A. Meijer, Y. W. Kong, W. T. Lu, A. Wilczynska, R. V. Spriggs, S. W. Robinson, J. D. Godfrey, A. E. Willis, M. Bushwitz
eIF4A

- Archetypal DEAD-box RNA Helicase
- Unwinds RNA helices, expends ATP
- Abundant
- Activity controlled by binding partners
Synergistic activation of eIF4A by eIF4B and eIF4G

Role in proliferation

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eIF4A regulators

• PDCD4
  - Binds and inhibits eIF4A
  - Pro-apoptotic
  - Tumour suppressor

Crystal structure of the eIF4A–PDCD4 complex

- mTOR kinase activation
  - eIF4B-P
  - PDCD4-P
  - Activity
  - Degradation

ª PDCD4 and eIF4A are regulated by mTOR
ª PDCD4 targeted by miR-21

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Summary

Translation is regulated, primarily at initiation.

Different 5’ UTRs have different amounts of inhibitory structure.

Helicase activity is central to translation initiation.

Helicase activity is largely defined by:

- Helicase eIF4A
- Activator eIF4B
- Repressor PDCD4
Questions

1. Is mRNA helicase activity altered in breast cancer?
2. Does mRNA helicase activity affect the cancer cell phenotype?
3. Are there gene-specific effects of helicase dysregulation?
4. Is mRNA helicase activity related to survival?
1. Is mRNA helicase activity altered in breast cancer?
mRNA helicase activity varies in cell lines
Helicase activity varies in tumours

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2. Does mRNA helicase activity affect the cancer cell phenotype?
Helicase expression is related to tumour cell proliferation

Trend P<0.001

Trend P<0.001

Trend P<0.001

Trend P<0.001
PDCD4 expression is negatively related to clinical aggression.

Trend: P<0.001

Trend: P=0.006

Trend: P<0.001

Trend: P<0.001
Helicase activity limits cell growth
Helicase activity influences the cell cycle
3. Are there gene-specific effects of helicase dysregulation?
Measuring translational control

Inactive mRNA

Monoribosome

Polyribosome

Sucrose gradient
Polysome Profiling

Sucrose gradient

Fractions

Pool polysomal/subpolysomal fractions

Microarray/RNASEq Analysis
eIF4A knockdown affects global translation in MCF7
To identify helicase-regulated genes...

Å Knockdown eIF4A in a cell model (MCF7)
Å Separate polysomal and subpolysomal pools of mRNA
Å Quantify
  ï Illumina microarray
  ï RNAseq (Solexa)
Å For each mRNA detected, calculate
  ï Change in polysomes with eIF4A knockdown
  ï Change in subpolysomes with eIF4A knockdown
  ï Plot
mRNAs that respond to eIF4A knockdown

Translationally eIF4A-independent

Translationally eIF4A-dependent
eIF4A-dependent mRNAs have more GC-rich 5’UTRs
Helicase-dependent mRNAs

- 175 mRNAs
- Enriched for protein families
  - G-proteins (P=6e-06)
  - Cyclins (P=0.003)
  - Kinases (P=0.05)
- Enriched for KEGG categories
  - MAPK (P=0.008)
    - ADCY3 ADRBK1 AKT1S1 ARAF BCKDK CCND3 CCNK CDC42BPB CSNK1E DVL3 EPHB4 GNAI2 GPS2 HIPK3 KIAA1804 MAPK3 MAPKAPK2 MARK4 MKNK2 NCK2 PKN1 PRKACA SMAD2 TESK1 TGFB1
  - Cancer-related KEGG categories
  - Pancreatic, colorectal cancer signatures
Helicase-independent mRNAs

- 47 mRNAs
- Enriched for protein families
  - Zinc finger proteins (P=0.01)
- Enriched for KEGG categories
  - Ribosome components (P=0.003)
  - Oxidative phosphorylation (P=0.04)
- Ribosome proteins have short unstructured 5’UTRs
Are there also effects upon the transcriptional programme?

- Knockdown eIF4A
- Profile total mRNA
Total mRNA profile

- Elevated in ‘high helicase’ state:
  - Cell surface receptor linked signaling pathways (P=0.0007)
  - Cell motility (P=0.0007)
  - Anti-apoptosis (P=0.003)
  - Various carcinoma signatures
4. Is mRNA helicase activity related to survival?
Helicase activity predicts poor outcome in ER-negative breast cancer

Multivariate Cox model (3yrs):
(including grade, nodes, size, HER2)
HR=\textbf{1.6} (1.1-2.4) P= 0.015

Multivariate Cox model:
(including grade, nodes, size, HER2)
HR=\textbf{1.5} (1.0-2.1) P= 0.027
PDCD4 predicts good outcome in ER-positive breast cancer

Multivariate Cox model:
(including grade, nodes, size, HER2, Aurora kinase)

$HR=0.7$ (0.6-0.8) $P<0.001$
Survival models suggest:

- Helicase activity is a powerful predictor of outcome in breast cancer
- Identifiable poor survival groups by their expression of helicase
  - Dependence on helicase?
- Different effects in ER+ and ER- tumours
- Highly independent of other known clinicopathological variables
- Always independent of measures of proliferation
  - Implies that survival value is exerted by effects upon other aspects of the tumour phenotype
A model

- Altered eIF4A/eIF4B/PDCD4 expression
- Elevated helicase activity
- Derepressed translation
  - Globally enhanced protein synthesis
  - Enhanced translation of mRNAs with structured 5’ UTRs
  - Generalised upregulation/’coarsening’ of intracellular signalling
- Proliferation
- Malignant phenotype
Final thoughts

• Translation factor activity is a potent predictor of outcome in breast cancer
• Similar mechanisms are likely to apply in other malignancies
• Upstream events?
• Relationship to micro-RNA expression/dysregulation
  • Does helicase upregulation facilitate miR oncogenesis?
  • Are helicase upregulation and miR loss equivalent?
• Helicases are ‘druggable’ – preclinical trials promising
• The dominant paradigm...
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