Propojení výuky oborů Molekulární a buněčné biologie a Ochrany a tvorby životního prostředí OPVK (CZ.1.07/2.2.00/28.0032)

A BIG – HEARTED MOLECULE

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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

INTRODUCTION

- Blockade of the enzyme PDE9 prevents degradation of the molecule cyclic GMP which has been shown to protect against heart failure.
- The finding indicates that PDE9 inhibition might be a drug target for treating this condition.

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Heart failure

- Heart can´t pump sufficient blood to meet the needs of the body
- Leading cause of death worldwide
- Important risk factor for heart failure is persistent high blood pressure, because it increases heart‘s workload → cardiac hypertrophy
- Heart releases natriuretic peptide hormones → lower blood pressure and exert direct cardioprotective effects
- But in heart failure natriuretic-peptide signalling is attenuated
- Lee *et al.* demonstrate that restoration of this signalling may be a useful strategy for treating heart failure.

Cyclic guanosine monophosphate (cGMP)

- Numerous studies have shown that an intracellular molecule called cyclic GMP (cGMP) provides protection from high blood pressure and cardiac disease, in part by activating the enzyme cGMP-dependent protein kinase type I (PKGI), which modulates the activity of many targets protein.

HORMONES

- In this cells cGMP production is promoted by four hormones, acting through three receptor proteins found in different subcellular locations.

1. Gaseous hormone nitric oxide (NO) → stimulates guanylyl cyclase (sGC) receptor
2. Atrial natriuretic peptide (ANP) → activate a guanylyl cyclase type A (GC-A) receptor
3. B-type natriuretic peptide (BNP) → activate a guanylyl cyclase type A (GC-A) receptor
4. C-type natriuretic peptide (CNP) → binds to a membrane-spanning guanylyl cyclase type B (GC-B) receptor

HORMONES

- NO and CNP are secreted from endothelial cells that line the blood vessels
- ANP and BNP are produced by cardiomyocytes themselves
- Opposing the action of these hormones, the phosphodiesterase enzymes PDE5 and PDE9 degrade cGMP by hydrolysis and so regulate the duration, amplitude and spatial distribution of cGMP signalling within cardiomyocytes.

PDE5 INHIBITION

- The accumulating evidence of cGMP's role in the heart led to an interest in drugs that might enhance cGMP signalling → much attention has focused on PDE5 inhibition.
- Since 1998, three PDE5 inhibitors have been used to treat erectile dysfunction and pulmonary hypertension.
- Preclinical studies with one such inhibitor, sildenafil, showed dramatic cardiac antiremodelling benefits in animals, and these were partly confirmed in small human studies. But a large clinical trial in patients with heart failure reported disappointing results.

Research

- Lee *et al*. investigated the cellular distribution of PDE5 and PDE9 in cardiomyocytes
- PDE5 is found at contractile filaments called myofilaments, where it degrades cGMP produced through the NO–sGC pathway.
- But PDE9 is located near 'T-tubular' invaginations of the plasma membrane and mainly regulates cGMP produced by the ANP–GC-A pathway.
- The authors also found that PDE9 levels and activities, like those of PDE5, rise in hypertrophic cardiomyocytes, both in mice and in patients with heart failure.

RESEARCH

- Oxidative stress provokes a dysfunctional uncoupling of the NO-producing enzyme in patients with heart failure, leading to the production of noxious oxygen radicals instead of NO.
- Lee and colleagues reasoned that this effect, which attenuates cGMP production through the NO–sGC pathway, could explain the limited clinical effectiveness of PDE5 inhibitors. But natriuretic-peptide-driven cGMP synthesis is also compromised in the cardiomyocytes of patients with heart failure — GC-A receptors become desensitized, and the cells secrete large amounts of precursors to ANP and BNP, which are not properly processed and so are poorly active.
- The authors therefore propose that inhibiting PDE9 is an attractive alternative to inhibiting PDE5.

Research

- Mice do not normally develop high blood pressure or cardiac hypertrophy, so the researchers surgically constricted the aorta, which carries blood away from the heart, thereby artificially enhancing cardiac 'afterload'.
- → these hearts were 60% larger.
- Remarkably, genetic or pharmacological inhibition of PDE9 (using a compound called PF-04447943) prevented and even reversed existing heart failure in these mice.

Research

- The authors' suggestion that modulating the pool of ANP- or BNP-derived cGMP can benefit patients with heart failure is supported by research showing that inhibiting neprilysin (a peptidase enzyme that degrades ANP, BNP and other hormones) enhanced endogenous ANP and BNP levels, and reduced the risks of hospitalization and death in patients with heart failure

In conclusion

- Other strategies for treating heart failure are also worthy of consideration. For instance, one approach under investigation is the use of synthetic ‘designer’ natriuretic peptides. The CNP–cGMP pathway and corin, an enzyme that activates cardiac ANP, may also represent targets for heart-protecting therapies.
- PF-04447943 is being tested in clinical trials for neurocognitive diseases and seems to be well tolerated in humans. Lee and colleagues’ exciting observations in mice, when considered together with the fact that older patients with heart failure frequently exhibit cognitive impairment, support the exploration of PDE9 as a target for treating heart failure.

Thanks for your attention

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Embryo editing sparks epic debate

Sara Reardon

David Cyranoski

Ondřej Janča

http://www.nature.com/polopoly_fs/7.25710.1430236986!/image/for-web-P6800383-Coloured_SEM_of_human_embryo_at_8-cell_stage-SPL.gif_gen/derivatives/landscape_630/for-web-P6800383-Coloured_SEM_of_human_embryo_at_8-cell_stage-SPL.gif

Chinese were the first

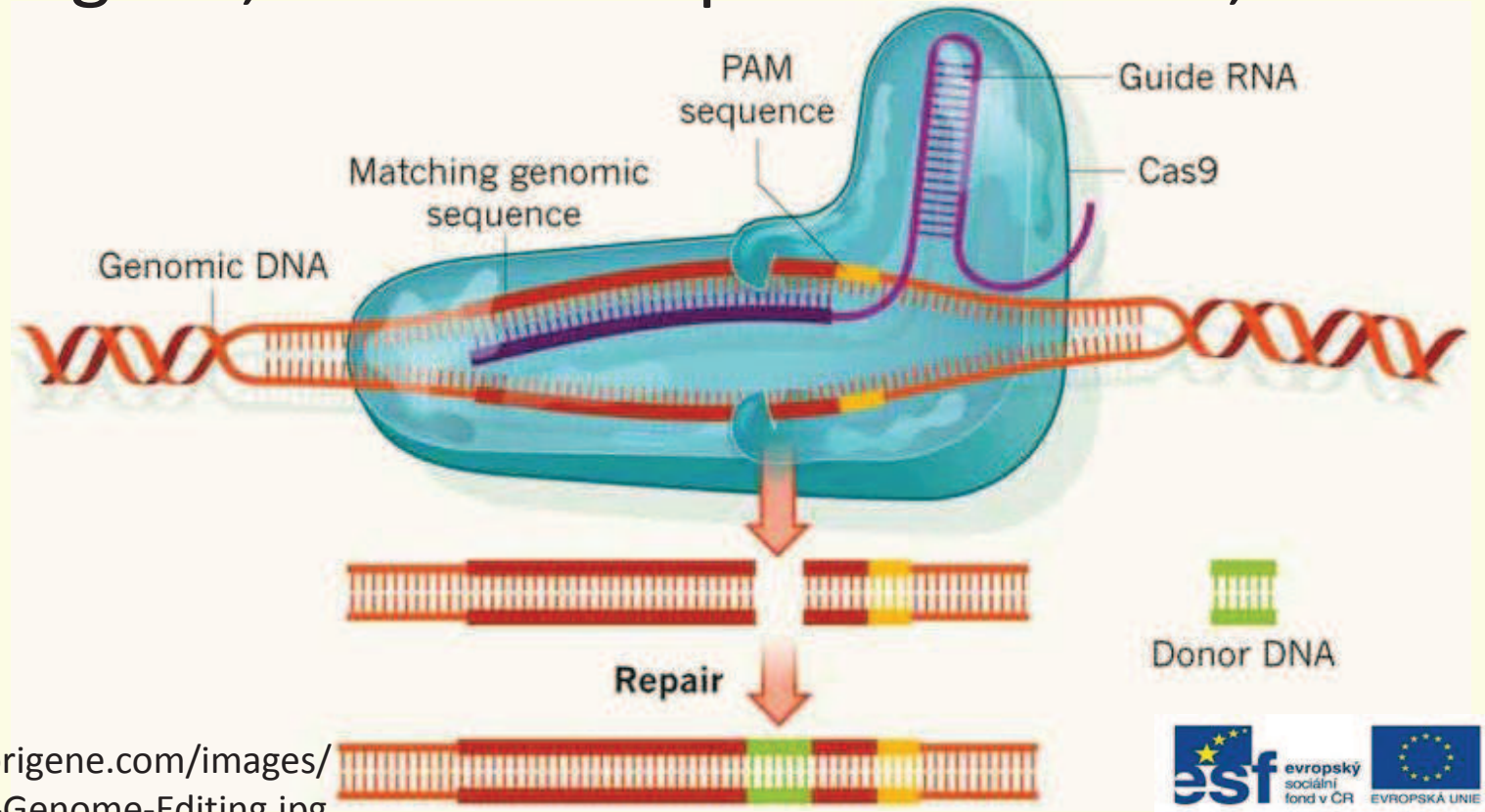
- Junjiu Huang et al.
- **CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes**
- Protein & Cell April 2015

available online:
http://link.springer.com/article/10.1007/s13238-015-0153-5/fulltext.html

picture URL: https://scholar.google.com/citations?view_op=view_photo&user=tbM39mMAAAAJ&citpid=1

The article

- **CRISPR/Cas9**
- cut DNA in human embryos, repair by new DNA
- on gene, that causes β-thalassaemia, when mutated



http://www.origene.com/images/service/CAS9-Genome-Editing.jpg

Ethical concerns

- used non-viable embryos obtained from fertility clinics
- eggs fertilized by two sperms
- **polyploidy** → could not result in a live birth

Reactions

procedure could eradicate devastating genetic diseases before a baby is born

X

genetic changes to embryos (germline modification) are heritable and could have an unpredictable effect on future generations

&

slippery slope towards unsafe, unethical or non-medical uses of the technique

Obstacles

- Rate of success only about 5%.

Nr. of embryos	Phase of experiment
86	injected with CRISPR/Cas9 and other molecules
71	survived 48-hours incubation
54	genetically tested
28	successfully spliced
4	contained desired genetic material

- Off-target mutations:
  - in other parts of exome
  - way more than in animal cells
  - might be caused by using polyploid cells

Moratorium

- technichal issues (RoS, mutations)
- immature technology

- outdated technique
- it was ethical with pluripotent stem cells
- only way to adress some questions about early human development

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Probable conclusion

- The technique will not be available for clinical use until ethical and safety concerns are worked out.
- There won‘t be a moratorium on the research of germline modification.

Next steps

- Huang‘s team plans to work out how to decrease the number of off-target mutations.
- the technique could answer basic scientific questions that have nothing to do with clinical applications:
  - altering developmental genes with CRISPR/Cas9 could help to reveal their functions
  - engineering specific disease-related mutations, which could then be used to produce models for testing drugs and other interventions for diseases

More to come

- researchers expect to see more gene-editing studies in human embryos
- “The ubiquitous access to and simplicity of creating CRISPRs creates opportunities for scientists in any part of the world to do any kind of experiments they want.”

Resources

- http://www.nature.com/news/embryo-editing-sparks-epic-debate-1.17421#/b1
- http://en.wikipedia.org/wiki/Polyspermy
- http://en.wikipedia.org/wiki/Thalassemia