A Gold Mine
Underground Against Cancer:
Hypoxia Tolerant Spalax Holds the Key for Treatment

Imad Shams, Irena Manov, Mark Hirsh, Assaf Malik, Mark Band, Aaron Avivi
Institute of Evolution University of Haifa, Haifa Israel

HYPOXIA

- Spalax survives oxygen levels as low as 3% (under these conditions rats die within 2-3 hours). For comparison, the level of free oxygen on top of Mt. Everest is ~8%
- Hypoxia is directly related to the most lethal diseases in the western world: heart and lung diseases, brain strokes, and cancer
- Spalax hypoxia-tolerance is directly related to its resistance to these diseases.
- Hence Spalax is also a promising model for clinical and pharmaceutical development for combatting and preventing these diseases.
- A long list of physiological parameters and genes are expressed differently in the subterranean Spalax as compared to aboveground mammals

VEGF EXPRESSION
A ratio of 1.4 between an abnormal growth and healthy tissue, is a marker for suspicious malignant development

Spalax specific amino acid changes within the p53 DNA binding domain mimic common mutations, arginine-lysine R174K and R209K, observed in tumors.

Relative transactivation of eight different human promoters as demonstrated in Luciferase Assay
**Spalax Heparanase**
- Heparanase is crucial for the progression of cancerous tumors and encouragement of metastases.
- Spalax heparanse expression is hypoxia-related, and exhibits unique splice variants.
- When we compared the effect of the native protein and the Spalax unique form in mice models, we found that one of Spalax’s splice variant induces tumors which are 7 times smaller than the native protein (upper photo, lower row) as well as inhibiting the metastatic activity (lower photo, lower row; yellow ‘stains’).

**Spalax: A Highly Cancer Resistant Mammal**
- Example 1: 3MCA was used to induce fibrosarcoma injection. Of 3MCA injection, 4 months after 2 months after Tumor in Mice.
- Human and Mice cancer cells (liver, breast, lungs) that are fed with conditioned medium from Spalax fibroblasts are dying (upper panel; RED). Only 50% of them are still alive after a week. However, the same cancer cells survive (lower panel; BLUE) when fed with mice, rat, human and Acomys conditioned media (Acomys a wild rodent, like Spalax. Used to avoid criticism that laboratory mice and rats are no longer ‘normal animals’).

**Spalax normal fibroblasts kill human cancer cells grown in co-culture.**
- This ability is unique to Spalax and directed only towards cancer cells. Mice, rats and another wild, field-captured rodent, an above-ground ‘relative’ of Spalax — the spiny mice do not have this capability.

**Spalax: A Highly Cancer Resistant Mammal**
- Example 2: DMBA/TPA induces skin cancer. After 10 days Necrotic lesions develop. After 3-4 months all mice suffered from the expected tumor. All Spalax healed (even if the treatment continued for 6 months) one of Spalax’s splice variants.

**Conditioned Medium from Normal Spalax Fibroblasts Kills Human Cancer Cells**
- Human and Mice cancer cells (liver, breast, lungs) that are fed with conditioned medium from Spalax fibroblasts are dying (upper panel; RED) in their respective medium. However, the same cancer cells survive (lower panel; BLUE) when fed with mice, rat, human and Acomys conditioned media (Acomys a wild rodent, like Spalax. Used to avoid criticism that laboratory mice and rats are no longer ‘normal animals’). NOTEWORTHY: medium where Spalax normal cells grew, does not kill normal, healthy cells of human mice, rats, Acomys and Spalax.

**Effect of Spalax, rat and mouse normal fibroblasts on Spalax-derived fibrosarcoma colony formation.**
- (A) After 3 weeks colonies were counted. Effects of different fibroblasts on colony formation are shown.
- (B) Colony numbers and cumulative total colony area (µm²) were calculated to demonstrate the effects of the fibroblast monolayer on cancer cell colony formation and growth.
HepG2 cells grown under medium conditioned by Spalax or rat fibroblasts

Decreased cancer cell viability and morphological changes (swelling, rounding, detachment),
Disturbed cell cycle progression,
Chromatin condensation (empty arrows) and fragmented nuclei (white arrows)
Mitochondrial fragmentation
All suggest apoptotic modes of cancer cell death.

Summary and Future Goals

Our results indicate that Spalax may be THE missing experimental organism to contribute progress towards human cancer treatment.

Our immediate goal is to identify those substance(s) secreted only by Spalax cells, specifically targeting cancer cells.

To pinpoint the unique component(s) or receptors on cancer cells that interact with these factors.

This will lead to the recognition of the molecular-biochemical pathways responsible for the interaction, resulting in cancer cell death.