

**Questions and Answers for the SDH Webinar (Answered by Dr. Eyal Gottlieb)**

1. What research is being done currently on SDHC epimutant GIST and what is most current info known about that type of SDH deficient GIST. Most of the studies are genetic studies – i.e. characterizing more events and potential causes for these event. The question here should be whether demethylation processes (or more specifically DNA methyl transferase inhibitors) should be explored therapeutically. This is also relevant to all SDH tumors as the consequence of SDH loss of function is hyper-methylation due to the accumulation of succinate and the consequent inhibition of demethylases. *Azacitidine* and its derivatives (e.g. *Decitabine*) are approved drugs and they may be an important avenue to explore therapeutically.
2. Have you observed SDH families having non tumor symptoms that could be related to the SDH “major energy and metabolic crisis” (Brain fog, headache, fatigue, exercise intolerance or others?). Can you describe the hearing loss associated with SDH families? First, I am neither a geneticist nor clinician – and I do not work with families. There is however a well-documented bi-allelic germline mutation syndrome that leads to a general metabolic deficit in the whole body of the affected patients. Here the ‘bad’ gene comes from both parents – so both copies are not fully functional in every cell in the body. It is different than the mono-allelic germline mutation that are associated with somatic (no germline) loss of the second allele and with cancer formation. One major difference MAY be the fact that the mutations associated with the bi-allelic germline mutations of SDH are not a complete loss of function (it is likely that embryogenesis cannot occur without at least partial SDH activity).
3. Is SDHD cancer? Are bilateral carotid body tumors, (paragangliomas) cancer? SDHD is a name for a gene and its gene product (protein), while cancer is a disease of cells growing and spreading uncontrollably. However, cancer can occur due to loss of some genes (like SDHD). Further clarification: not all cell growth phenomena are considered cancer. Benign tumors are not ‘officially’ cancer as they do not invade and spread. Some of them can become cancerous though, and some non-cancer benign tumors can have major effects and even cause death. Paraganglioma can be benign or malignant (cancerous), but even benign paraganglioma can have major effects on blood pressure, hormonal (e.g. adrenaline) levels etc. – so it is not a trivial disease.
4. Can you explain the differences between SDH genes and what makes them so different? (SDHA, SDHB...)  
SDH is an enzyme (a unit, typically made of protein(s) that enable biochemical reactions). SDH is a complex made of 4 proteins (A-D), each one with different contributions to the overall SDH activity. Each one of the subunits is essential for SDH function, hence, biochemically-speaking, the loss of function of any of the subunit will have a similar consequence – which is the complete loss of SDH activity. So, while the 4 SDH subunits differ structurally and functionally from each other, the outcome of their loss is rather similar. There is insufficient understanding (or evidence) for a direct link between the different SDH mutations and the different cancer phenotypes. It is possible that only the overall loss of SDH provides the pro-oncogenic function. The recorded differences between different mutations and different appearances of the disease can be either stochastic, or can be attributed to some partial residual (or altered) functions of SDH associated with specific mutations. It is also possible that the location of the specific SDH gene on the chromosome is particularly relevant because a partial chromosomal deletion is typically associated with the second (somatic) genetic hit on the SDH gene. So, other genes may be co-deleted (at least on one chromosome). The potentially co-deleted genes will be

different in SDHA, B, C or D tumors because those SDH genes are located on different regions of the chromosome. But I must stress that all of that is hypothetical.

5. How common are familial cancers associated with inherited SDH mutations. I do not know the exact statistics, but these are considered uncommon, or rare events. Familial cancers (derived from ONE germline mutation – so called mono-allelic disorders) are uncommon in general, and of these, SDH is not the major contributor.
6. When someone is born with a germline SDHB mutation, Do you think there is a second mutation involved for tumors to grow whether epigenic or another faulty gene? Most certainly! Cancer is a complex genetic disorder.
7. Is the build up of succinate because of the broken SDHx cycle the tumor driver? Succinate is very likely to play a major role (as I will explain in the seminar). But it does not mean that it is the only mechanistic link between SDH loss and cancer.
8. I've read "3-Nitropropionic Acid Is a Suicide Inhibitor of Mitochondrial Respiration" thus could those with SDHx gene mutations who've had exposure to one of these: black molds,, fungi, food molds (such as found on corn, peanuts) be one the second SDHx allele mutation occurred and thus tumors followed? This is not likely. The second (and third etc...) hits are genetics/epigenetics and any natural (endogenous) or exogenous mutagen may cause it. No need to search for chemicals that may inhibit respiration. While we cannot exclude it, currently, there is no good rational to include such a theory.
9. I've been told SDHx mutation tumors have nothing a chemo agent can "target" are you working on anything that is hopeful to change that reality? Indeed, there are no 'direct targets' that are known to be unique for SDH-deficient tumors. However, 'targeted therapy' is in use for SDH-deficient tumors – e.g. anti-angiogenesis (anti-VEGF). In general, it is more difficult to identify molecular targets in cancers derived from a loss of 'tumor suppressor genes'. Here the search is for targets that become essential specifically in the cancer cells due to the loss of SDH activity. These are called 'induced essentiality', 'synthetic lethality' and 'non-oncogene addiction' targets. I will discuss it in my seminar. And yes, I am working on it very hard...
10. Do you think supplements such as ubiquinol, l-methylfolate, Vitamin C, riboflavin could help someone with a broken Krebs's Cycle? I am not a great believer in such an approach. It is not trivial to fix a broken TCA (Krebs) Cycle, and certainly not with co-factors. Of those mentioned, there is a slight rational in using vitamin C, because dioxygenases, the enzymes that are inhibited by succinate in SDH-deficient tumors, are using vitamin C as a co-factor, and maybe excess vitamin C will help retaining better activity of these succinate-inhibited enzymes. However, typically vitamins are not a limited factor in enzymatic functions, hence increasing their concentration would not necessarily (in fact will likely not) enhance enzymatic activity.
11. Do you have any working mouse models or cell lines? If so would you be willing to see if Dr. Meiri could find a strain/formula that could keep tumors from growing? I have made cell lines and mice with SDHB-deficiency. These are published and I make them available to anyone who ask. I also screened for small molecules (potential drugs) on these cells, and I am certainly looking for therapeutic approaches for this disease. However, a reality check comment: killing cells in tissue culture (in-vitro) is much easier than treating patients. For an effective molecule in-vitro to become a drug, there is a need to develop from it



molecules that can sustain their levels and prolonged activity on the tumor in the body, while sparing side effects (toxic outcomes) of the treatment on healthy tissues. So, there is a huge gap between screening compounds on cells and developing drugs.

12. Is Gleevec effective for SDHA GIST? Gleevec is a good inhibitor of active KIT, an oncogene that is associated with GIST. However, most SDH GIST do not carry active mutant KIT.
13. The phase trials currently being offered to SDH GIST patients: Do you feel any of these agents could curtail tumor growth?
  1. a demethylizing agent (I also read that a low dose of a demethylizing agent might work); Discussed in Q1 above regarding *Azacitidine* and *Decitabine*
  2. TKIs; Particularly inhibitors of VEGFR
  3. Temodar, an Alkylating antineoplastic agent; Alkylating agents, such as Temodar (Temozolomide), cause DNA damage, and maybe, the hypermethylated DNA is more refractory to DNA repair – hence more sensitive to DNA damage. The potential sensitivity to Temozolomide can be assessed by the level of the enzyme methylguanine-DNA methyltransferase (MGMT) in the tumors. MGMT correct the damage caused by the drug. Interestingly, MGMT can be silenced by methylation, and this can indicate sensitivity to Temozolomide. So, due to the hyper-methylated profile of SDH-deficient tumors, there is a rational to test for MGMT low expression and with that, to potential sensitivity to alkylating agents. However, these are all indirect links, and while the rational is potentially there, there is more to study before one can provide a clear answer.
  4. a HIF2b inhibitor; Certainly HIF2 is a very interesting target to explore, particularly in paraganglioma/pheochromocytoma where the genetic evidence for HIF involvement is stronger. There is an ongoing clinical trial with such an inhibitor - to my knowledge, with kidney cancer (and not SDH related tumors).
  5. a PARP1 inhibitor: The rational here is somewhat indirect (as with the Temozolomide) and feels a bit opportunistic. However, should be (and to my knowledge, it is) tested in a clinical setup.