Succinate Dehydrogenase Deficiency in Sporadic Pituitary Adenomas: A Potential Mechanism for Tumorigenesis

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ABSTRACT

In order to determine whether succinate dehydrogenase (SDH) deficiency plays a role in sporadic, non-familial pituitary adenomas, we analyzed 80 pituitary adenomas for SDH deficiency from patients without familial tumor syndromes or without known SDH deficiency-associated neoplasms. SDH deficiency was determined by immunohistochemical (IHC) stains for SDHB since the loss of any of the four SDH subunits results in the loss of SDHB expression. Three pituitary adenomas showed complete loss of SDHB staining, and of these two also showed loss of SDHA staining. We further characterized these adenomas by looking at Ki67, IGF1R, and 5-hmC levels via IHC. SDH*x*-deficient (non-SDHA deficient) tumors had a Ki67 proliferation index higher than non-SDH deficient pituitary tumors while SDHA-deficient tumors had Ki67 indices similar to non-SDH deficient tumors. IGF1R IHC staining was similar across all subsets. All SDH-deficient subtypes showed a loss of 5-hydroxymethylcytosine nuclear IHC staining. These findings suggest that SDH deficiency promotes tumorigenesis of pituitary adenomas through accumulation of succinate resulting in changes in the epigenome, specifically resulting in a hypermethylated state.

INTRODUCTION

Pituitary adenomas are relatively common neoplasms of the central nervous system found in approximately 16% of the population. Among all intracranial neoplasms, pituitary adenomas account for 10% of pathological new growths and are the most common neoplasms within the sella. These tumors are categorized based on size, invasiveness, secretory capacity and specificity, and are almost invariably derived from the adenohypophysis. Treatment is primarily surgical.

The genesis of pituitary adenomas is still largely unresolved despite the association of these tumors with syndromes that have known progenitor mutations. Several families with germline mutations in the genes encoding SDH subunits have been associated with pituitary adenomas and SDH deficiency is pro-oncogenic in a variety of other tumors, including extra-adrenal paragangliomas, a subset of gastrointestinal stromal tumors, pheochromocytomas, and renal cancers. Familial and genetic pituitary adenomas, however, account for only a small percentage of all pituitary adenomas (~5%). While SDH-deficient tumors typically occur in the setting of germline mutations, SDH-deficient tumors can occur sporadically.

The proposed mechanisms for tumorigenesis in SDH deficiency are loss-of-function mutations in or epigenetic silencing of SDH subunit genes that then cause the intermediate metabolite succinate to accumulate. Succinate build-up can result in a pseudohypoxic state within the cell which, through HIFα, upregulates expression of angiogenic factors and enzymes that aid in cell survival. Another possible mechanism of tumorigenesis related to succinate accumulation is inhibition of α-ketoglutarate-dependent dioxygenases, particularly the ten eleven translocaton (TET) family of 5-methylcytosine (5mC) hydroxylases, which produces epigenetic alterations. In particular, SDH-deficient tumors have elevated hypermethylation compared to non-SDH deficient counterparts, suggested by the lower levels of 5-hydroxymethylcytosine (5-hmC).

METHODS

The pituitary adenomas analyzed were from patients without familial tumor syndromes or without known SDH deficiency-associated neoplasms. Of the 80 cases, 40 were females and 40 were males. The mean age was 52.3 years old (range 14-85).

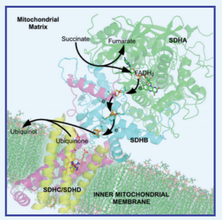


Figure 1: The SDH complex. There are four subunits: A, B, C, and D. The A and B subunits are hydrophilic and reside in the mitochondrial matrix. The C and D subunits are hydrophobic and are anchored in the inner mitochondrial membrane. The A subunit serves as the binding site for succinate.

METHODS

All SDH-deficient pituitary adenomas were described clinically as large. The two SDHA deficient adenomas were also invasive, with one growing into the nasal sinuses and the other extending into the cavernous sinus. Tissue was fixed in formalin and embedded in paraffin blocks. 5 micron-thick sections of representative pituitary adenomas were punched out of these blocks and assembled into tissue arrays with 40-45 samples per slide (Figure 2). Arrays were analyzed by IHC for expression of SDHA, SDHB, Ki67, IGF1R, and 5-hmC by standard methods (Figure 3).

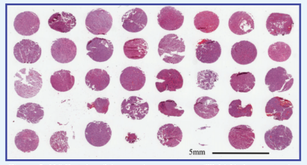


Figure 2: H&E stain of tissue microarray one (TMA1) demonstrating the format of these arrays. Empty locations represent areas of lost tissue.

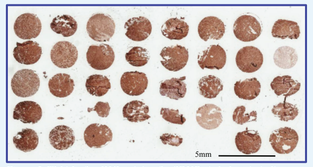
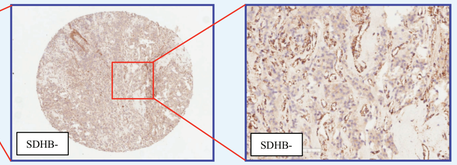


Figure 3: TMA1 after IHC staining for SDHB. A dark stain indicates presence of SDHB and thus functioning SDH. Light to no staining indicates SDHB absence and by extension SDH deficiency.

RESULTS

SDH IHC

Figure 4: Tissue microarray one IHC for SDHB. **A**: 27 yo male with a 'large' pituitary adenoma. **B**: 56 yo male with an invasive pituitary adenoma.



Figures 5 & 6: Note the absence of the normal granular mitochondrial stain for SDH in this tissue sample from a 56 yo male. The fibrovascular framework is responsible for the scattered positive staining.

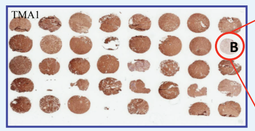


Figure 7 (8 & 9): Tissue microarray one IHC for SDHA. **B** from the SDHB IHC also stained negatively for SDHA. Control SDHA staining is similar to control SDHB staining (below) in its strength and pattern.

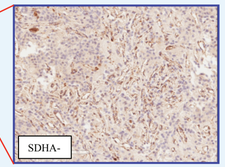


Figure 8: **B** from SDHB IHC showing no SDHA.

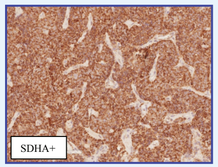


Figure 9: Control, non-SDH deficient SDHA IHC.

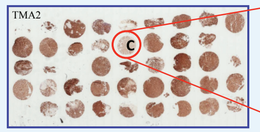


Figure 10 (11 & 12): Tissue microarray two IHC for SDHB and SDHA (not shown). **C**: 70 yo male with local invasion.

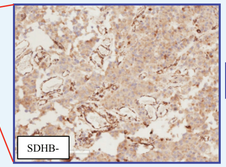


Figure 11: SDHB absence in tumor that extended into the cavernous sinus.

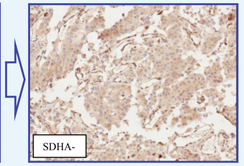
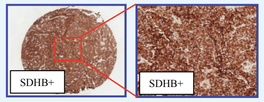


Figure 12: SDHA absence in the same patient.



Figures 13 & 14: Control, non-SDH deficient staining for SDHB is diffuse, dark, and granular.

RESULTS

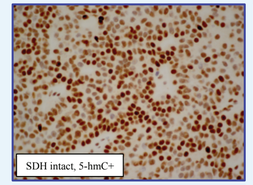
Ki67 IHC

Tumor Group	Ki67 proliferation index
Non-SDH deficient (n=77)	1.3%
SDHA deficient (n=2)	0.9%
SDH*x* deficient (n=1)	5.5%

Table 1: The Ki67 proliferation index reflects the number of cells actively in the cell cycle and may reflect growth rate and growth potential of a tumor. The two SDHA-deficient pituitary adenomas had Ki67 proliferation indices similar to other non-SDH deficient pituitary adenomas. The SDH*x*-deficient pituitary adenoma (adenoma with a defective B, C, or D subunit), on the other hand, had an abnormally increased Ki67 proliferation index. IGF1R staining of SDH-deficient adenomas was in the range of IGF1R staining of non-SDH deficient adenomas (data not shown). Analysis of additional cases is necessary to determine if these differences and similarities are accurate.

5-hydroxymethylcytosine (5-hmC) IHC

Figure 15: Positive nuclear staining for 5-hmC by IHC is noted in a pituitary adenoma without SDH subunit deficiencies and intact SDH.



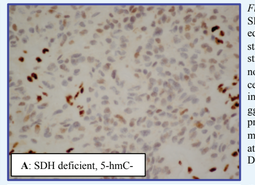
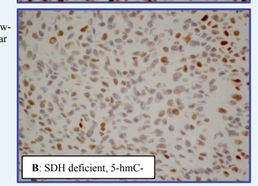


Figure 16: IHC for 5-hmC in SDH-deficient adenoma **A** showed loss of and decreased nuclear staining of 5-hmC. The intact, strong nuclear 5-hmC staining is noted in intratumoral endothelial cells. The loss of nuclear 5-hmC in this pituitary adenoma suggests that SDH deficiency may promote pituitary adenoma formation by altering the methylation of cytosine residues within DNA and histone proteins.

Figure 17: IHC for 5-hmC in SDH-deficient adenoma **B** showed loss of and decreased nuclear staining of 5-hmC.



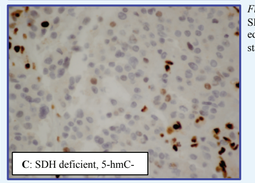


Figure 18: IHC for 5-hmC in SDH-deficient adenoma **C** showed loss of and decreased nuclear staining of 5-hmC.

CONCLUSIONS

1. SDH deficiency may account for tumorigenesis in approximately 3.8% of sporadic pituitary adenomas.
2. Analysis of additional cases and molecular analysis will be a necessary next step to determine clinical differences in the behavior of SDH-deficient pituitary adenomas compared to non-SDH deficient pituitary adenomas, and the differences in behavior of the distinct subtypes (SDHA, SDHB, SDHC, and SDHD) of SDH-deficient pituitary adenomas.
3. Accumulation of cellular succinate resulting from SDH deficiency may cause pituitary adenomas due to abnormal DNA methylation, in particular hypermethylation.
4. Increased understanding of how SDH deficiency leads to pituitary adenomas may lead to development of targeted therapy for this subtype of pituitary adenoma with the ultimate goal of replacing surgical treatment.

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Image credit for SDH: http://en.wikipedia.org/wiki/Succinate_dehydrogenase