Integrative Cancer Science and Therapeutics

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Hydroxyindole O-methyltransferase controls cancer growth and metastasis

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Commentary

5-methoxytryptophan (5-MTP) was identified as a new tryptophan metabolite which suppresses cyclooxygenase-2 (COX-2) expression, cancer cell migration and epithelial mesenchymal transition [1,2]. 5-MTP is synthesized in human fibroblasts via two enzymatic steps: tryptophan hydroxylase-1 (TPH-1) which catalyzes conversion of L-tryptophan to 5-hydroxytryptophan (5-HTP) and hydroxyindole O-methyltransferase (HIOMT) which catalyzes transfer of methyl group from a methyl donor to 5-HTP to form 5-MTP. We reported that A549 cancer cells produce very low level of 5-MTP due to defective expression of HIOMT. Overexpression of HIOMT by stable transfection of a truncated HIOMT isoform (HIOMT 298), in A549 cells restores 5-MTP production accompanied by reduced cancer cell migration in vitro and slower tumor growth and fewer lung metastatic nodules in a xenograft murine tumor model [3]. This commentary will address the HIOMT isoforms which catalyze 5-MTP vs. melatonin biosynthesis.

HIOMT (EC 2.1.1.4) was identified in pineal glands over a half century ago as an enzyme catalyzing transfer of methyl group from S-adenosylmethionine (SAM) to N-acetyl-5-hydroxytryptamine (N-acetyl-serotonin) to form melatonin (N-acetyl5-methoxytryptamine) [4]. As it catalyzes methylation of N-acetylserotonine, HIOMT has been commonly called N-acetylserotonin methyltransferase (ASMT). HIOMT was subsequently purified from pineal tissues [5] and its cDNA was cloned from pineal tissues of several species including bovine and human [6-8]. Three isoforms of HIOMT transcripts were detected in human pineal and retinal tissues, while only a single HIOMT transcript was detected in pineal glands of other species. Analysis of human genomic sequence revealed that human HIOMT is encoded by a single gene and the isoforms are derived from alternative splicing: full length mRNA codes for a 373 aa protein (isoform 373) while the spliced mRNAs code for a 345 aa (isoform 345) and 298 aa (isoform) proteins, respectively [8,9]. Human full-length mRNA contains exon 6 which shows sequence identity to LINE-1. Exon 6 is deleted in the short isoforms. Sequence comparison among species shows that human HIOMT 345 aligns best with HIOMT of other species whereas human isoform 373 is not detected in other species [9]. Structure-function analysis indicates that HIOMT 345 is active in catalyzing melatonin synthesis whereas neither isoform 373 nor isoform 298 is active [10]. By contrast, experimental results suggest that HIOMT 345 is inactive in 5-MTP synthesis while the truncated HIOMT 298 (both exons 6 and 7 are deleted) is active. Only HIOMT 298 mRNAs are detected in fibroblasts [3] as well as human endothelial cells and bone marrow-derived mesenchymal stromal cells (MSCs). A549 cancer cells express a low level of HIOMT 298. Cloning and sequencing reveal that the sequence of HIOMT 298 cloned from human fibroblasts and A549 cells matches 100% with that of ASMT 298 detected in human pineal cells [3].

The catalytic activity of HIOMT 298 was investigated by stable transfection of A549 cells with HIOMT 298 as well as HIOMT 345 and HIOMT 373. 5-MTP released into the medium was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS-MS). HIOMT 298 overexpression resulted in a large increase in 5-MTP whereas HIOMT 345 overexpression did not increase 5-MTP levels, suggesting that HIOMT 298 but not isoform 345 is catalytically active. Despite absence of HIOMT 373 expression, HIOMT 373 overexpression increased 5-MTP to an extent comparable to HIOMT 298 overexpression, suggesting that HIOMT 373 is also catalytically active in 5-MTP synthesis. HIOMT298-overexpressed A549 cells migrate sluggish compared to control as analyzed by transwell assay. Furthermore, tumor growth and lung metastasis of HIOMT 298 stably transfected-A549 cells implanted into subcutaneous tissues of SCID mice are significantly reduced when compared to those of control A549 cells. These data provide evidence to support that HIOMT 298 is active in controlling cancer growth and metastasis via 5-MTP production.

The catalytic action of HIOMT 298 involves transfer of methyl group from a methyl donor to OH of 5-HTP. It remains unclear whether like HIOMT 345 in melatonin synthesis, HIOMT 298 uses SAM as methyl donor for 5-MTP synthesis. It was reported that peptide fragment encoded by exon 7 is situated at SAM binding site [10]. Since exon 7 is deleted in HIOMT 298, SAM binding region would be too narrow to bind SAM. This raises a question whether HIOMT 298 may use a smaller methyl donor. Alternatively, the structure of HIOMT 298 may differ from HIOMT 345 in that substrate (5-HTP) binding site and methyl donor binding site are rearranged to accommodate SAM binding. Work is in progress to resolve HIOMT 298 structure-function by X-ray crystallography.

HIOMT 298 mRNA levels are very low in several cancer cell lines including A549, MCF-7, HepG3 and HT-29 cells. Cancer cells fail to utilize added 5-HTP to suppress COX-2 expression [3]. HIOMT 298 protein expression in several types of human cancer tissues is lower than that in normal tissues adjacent to cancer [10]. HIOMT expression in human cancer tissues appears to be heterogeneous. A fraction of colon and pancreatic cancer tissues (10-20%) exhibits normal level of

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expression while a majority of cancer tissues have a reduced level of expression. Importantly, HIOMT expression levels are correlated with the pathological grades of cancer [10]. The results suggest that HIOMT may potentially be a new biomarker of cancer. Given that 5-MTP is effective in preventing A549 cancer growth and metastasis in murine models, cancer HIOMT 298 level may be theranostic biomarker for 5-MTP chemoprevention. Further human studies are needed to evaluate this new approach of cancer prevention.

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References

1. Cheng HH, Kuo CC, Yan JL, Chen HL, Lin WC, et al. (2012) Control of cyclooxygenase-2 expression and tumorigenesis by endogenous 5-methoxytryptophan. *Proc Natl Acad Sci U S A* 109: 13231-13236. [Crossref]

2. Cheng HH, Chu LY, Chiang LY, Chen HL, Kuo CC, et al. (2016) Inhibition of cancer cell epithelial mesenchymal transition by normal fibroblasts via production of 5-methoxytryptophan. *Oncotarget* 7: 31243-31256. [Crossref]

3. Chen HL, Yuan CY, Cheng HH, Chang TC, Huang SK, et al. (2018) Restoration of hydroxyindole O-methyltransferase levels in human cancer cells induces a tryptophan-metabolic switch and attenuates cancer progression. *J Biol Chem* 293: 11131-11142. [Crossref]

4. AXELROD J, WEISSBACH H (1960) Enzymatic O-methylation of N-acetylserotonin to melatonin. *Science* 131: 1312. [Crossref]

5. AXELROD J, WEISSBACH H (1961) Purification and properties of hydroxyindole-O-methyl transferase. *J Biol Chem* 236: 211-213. [Crossref]

6. Ishida I, Obinata M, Deguchi T (1987) Molecular cloning and nucleotide sequence of cDNA encoding hydroxyindole O-methyltransferase of bovine pineal glands. *J Biol Chem* 262: 2895-2899. [Crossref]

7. Donohue SJ, Roseboom PH, Klein DC (1992) Bovine hydroxyindole-O-methyltransferase. Significant sequence revision. *J Biol Chem* 267: 5184-5185. [Crossref]

8. Donohue SJ, Roseboom PH, Illnerova H, Weller JL, Klein DC (1993) Human hydroxyindole-O-methyltransferase: presence of LINE-1 fragment in a cDNA clone and pineal mRNA. *DNA Cell Biol* 12: 715-727. [Crossref]

9. Rodriguez IR, Mazuruk K, Schoen TJ, Chader GJ (1994) Structural analysis of the human hydroxyindole-O-methyltransferase gene. Presence of two distinct promoters. *J Biol Chem* 2690: 31969-31977. [Crossref]

10. Botros HG, Legrand P, Pagan C, Bondet V, Weber P, et al. (2013) Crystal structure and functional mappin of human ASMT, the last enzyme of the melatonin synthesis pathway. *J Pineal Res* 54: 46-57. [Crossref]