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Expression Analysis of CYP27B1 in Tumor Biopsies and Cell Cultures

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Abstract. *There is evidence for alternative splicing as a prevalent mechanism both in normal human cells and cancer cells. Alternative splice variants of the gene encoding the human 25-hydroxyvitamin-D_3-1alpha-hydroxylase (CYP27B1) in human glioblastoma were recently reported. Using combined nested and touchdown PCR, full length CYP27B1 as well as several splice variants were cloned. Here, this approach was used to analyze the expression of CYP27B1 and its splice variants in normal tissues, tumor tissues and cell cultures. Expression of CYP27B1 was found in all tumor tissues. The expression of CYP27B1 was also found in several normal tissues including lung, kidney, placenta, thymus and stomach and in derived cell cultures. The expression of splice variants of CYP27B1 were detected in 1 out of 3 tested brain samples, with no evidence for the expression of normal CYP27B1.*

Pathways underlying the classic metabolism of vitamin D_3 have been well described. Hydroxylation to the biological active metabolite, 1α, 25-dihydroxyvitamin D_3 [1α25-OH_2D_3 or calcitriol] is mediated by CYP27B1 in the kidney. In addition to renal CYP27B1 expression, several studies report extrarenal expression of CYP27B1 in various human tissues (1, 2). Furthermore, several cancer cells show expression of the CYP27B1 gene, including human melanoma (3, 4) and non-small cell lung carcinoma cells (1).

Recent studies focused on alternative splicing that may cause tissue-specific variations in normal human cells (5) and diseased human cells. A database analysis showed that nearly two-thirds of human genes expressed splice variants in normal tissues (6). The biologically important role of P450 cytochromes in alternative splicing is also well documented (7, 8). Regulating the enzyme level by alternative splicing is considered to be a frequent feature of many P450 genes and may be the cause for tissue-specific variations (9). Gene amplification and mRNA splice variants of CYP27B1 in human glioblastoma were previously reported. Here, a comparative analysis of the expression of 1α-hydroxylase and its splice variants in normal tissue, tumor tissue and derived cell cultures is presented.

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Materials and Methods

The TX3868 cell line was established and cultured as described previously (11). HEK293 and HeLa cell lines were purchased from the ATCC. The tumor samples were kindly provided by the Institute of Pathology, Thorax Hospital, (Heidelberg, Germany) and were used with the consent of the patients. Isolation of total RNA and mRNA and nested-touchdown PCR were performed as described previously (10, 12)

Results and Discussion

The expression of the gene for the human 25-hydroxy-vitamin D_3-1α-hydroxylase (CYP27B1) in human glioblastoma multiforme (GBM) was recently reported (10). This study and our most recent analysis identified various splice variants of CYP27B1 (11). Here, the expression level and the expression pattern of CYP27B1 in other tumors (lung cancer, melanoma and cervical carcinoma) and in various normal tissues (lung, brain, liver, stomach, spleen, kidney, colon, thymus and placenta) were analyzed. An overview of the expression status of CYP27B1 and its splice variants in the samples analyzed is given in Table I.

Expression of CYP27B1 in lung cancer. RT-PCR (12) was used to analyze the expression of CYP27B1 in 4 lung cancer biopsies and their corresponding normal tissues. Expression of normal CYP27B1 was found in all the cancer samples as well as in all the normal samples. Likewise, various splice variants of CYP27B1 were found in each sample. Except for

Table I. *Expression status of CYP27B1 and its splice variants in the analyzed normal tissues, tumor tissues and derived cell cultures.*

Tested sample	Expression status	
Normal tissues & cell cultures	Normal CYP27B1	Splice variants of CYP27B1
2223 lung	+	+
2224 lung	+	+
2226 lung	+	+
2227 lung	+	+
brain 1	-	-
brain 2	-	-
brain 3	-	+
liver	-	-
lung	-	-
stomach 1	-	-
stomach 2	+	+
spleen	-	-
kidney	+	+
colon	-	-
thymus	+	+
placenta	+	+
HEK293	+	+
cancer tissues & cell cultures		
2223 lung tumor	+	+
2224 lung tumor	+	+
2226 lung tumor	+	+
2227 lung tumor	+	+
MeWo	+	+
SkMel28	+	+
HeLa	+	+

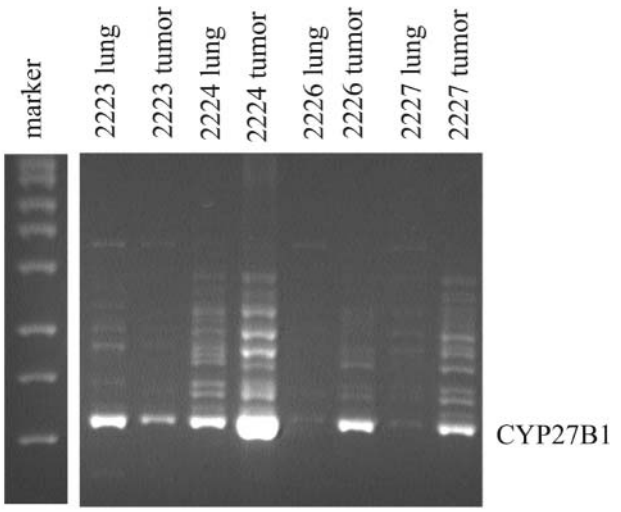

Figure 1. *Two-step RT-PCR (nested-touchdown PCR) with mRNA from tissues of lung cancer (2223, 2224, 2226 and 2227) and their corresponding normal tissues. The position of the normal CYP27B1 cDNA sequence is indicated.*

1 tumor sample, the level of expression of CYP27B1 in lung cancer was higher than in the corresponding normal tissue, as demonstrated in Figure 1, confirming previous findings of the expression of CYP27B1 in lung cancer (1). In addition, our data provide further evidence for the extrarenal activity of CYP27B1 that was previously reported for various human tumors, including melanoma, lung and placenta carcinoma (1, 2, 4, 13, 14).

Expression of CYP27B1 in normal human tissues. The expression of CYP27B1 was investigated in several normal human tissues including stomach, thymus, kidney, spleen, lung, placenta, colon and brain. Expression of normal CYP27B1 was found in 5 out of 9 samples, with a high expression in the kidney (Figure 2). These results are consistent with the observation that the kidney is the major location for the synthesis of calcitriol (15-17). Out of the remaining tissues positive for the expression of normal CYP27B1, the highest expression level was found in human placenta. These results are consistent with reports by Avila *et al.* (18), who detected the expression of CYP27B1 RNA in cultured human syncytiotrophoblast cells. The expression of CYP27B1 was also found in human thymus. These results are largely consistent with previous reports by Hollis (19), who provided indirect proof for CYP27B1 expression by showing interaction of the purported CYP27B1 with the bovine thymus vitamin D receptor protein.

Expression of CYP27B1 splice variants in human tissues. Human tissues that expressed normal CYP27B1 also expressed splice variants of this gene. In contrast, splice variants were also found in 1 out of 3 brain samples, which did not express the normal CYP27B1 (Figure 2). The splice variants found in the brain samples were different from those found in other human tissues. Specifically, a splice variant was detected at 2.2 kb and at 2.8 kb. Previously, Zehnder *et al.* (2) found expression of CYP27B1 in Purkinje cells of the cerebellum and in the cerebral cortex. Our studies did not provide evidence for the expression of normal CYP27B1 in human brain, but rather for the expression of splice variants of this gene in human brain. The expression of CYP27B1 and its splice variants also differed from the expression patterns of the other types of human cancer analyzed.

Expression of CYP27B1 splice variants in cultured cells. To lay the ground for further functional studies, the expression of CYP27B1 and its splice variants was analyzed in several

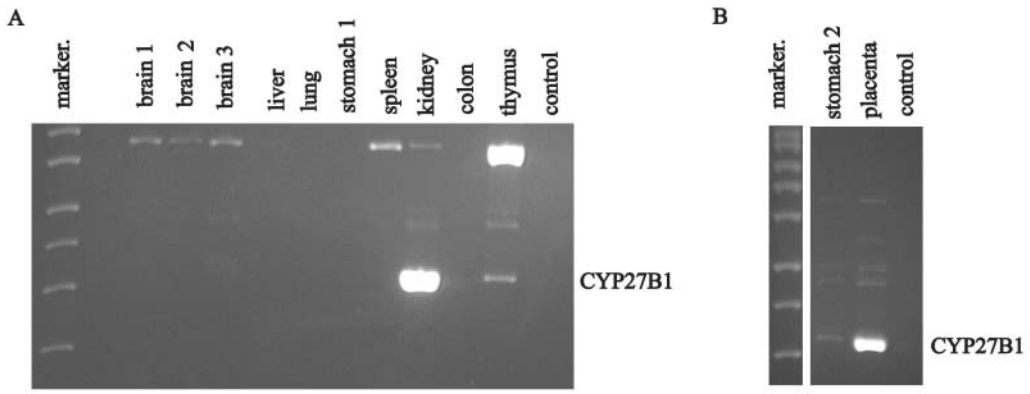

Figure 2. *Nested-touchdown PCR with mRNA from kidney and various extrarenal normal tissues. Tissues include (A) brain, liver, lung, stomach, spleen, kidney, colon, thymus and (B) stomach and placenta. The position of the normal CYP27B1 cDNA sequence is indicated.*

human cell cultures derived from melanoma, cervical carcinoma and embryonic kidney. The expression of normal CYP27B1 was verified in all the cell cultures analyzed. The melanoma cell line SkMel28 showed the highest level of expression of normal CYP27B1, while the embryonic kidney cell line showed a lower expression level. These results are inconsistent with previous reports demonstrating the major synthesis of CYP27B1 in the kidney. Furthermore, the lowest expression level of normal CYP27B1 was observed in the cervical carcinoma cell line. In addition, this cell line showed the lowest number of splice variants among the tested samples. Our data support previous studies that revealed the expression of CYP27B1 in HeLa cells and keratinocytes (20-22). This approach did not, however, allow quantitative measurements, due to the high product variety.

The overall pattern of splice variants in the melanoma cell lines differed from the pattern found in the remaining cell cultures and tissues analyzed. Specifically, the melanoma cell lines showed a highly-expressed splice variant at 1.8 kb, especially in the case of the melanoma cell line SkMel28 (Figure 3), while most cell lines showed the highest expressed splice variants between 2.8 and 3.3 kb (Figures 2 and 3). The splice variant at 1.8 kb was identified as Hyd-V2, which was previously reported to be expressed in GBM (11). Notably, the renal cells (HEK293) showed one of the highest expression levels of the normal 1,α-hydroxylase and a low expression level of the variants between 2.8 and 3.3 kb.

The mechanism of alternative splicing is considered to have potential as a diagnostic marker for cancer (23). In addition, splice variants are likely to play a crucial role in the regulation mechanism of CYP27B1. The expression of alternative splice variants may be associated with a lower expression level of normal CYP27B1 (24). We found that the majority of CYP27B1 splice variants was not degraded but was probably translated into proteins (11). The majority of these proteins, however, do not show enzymatic activity, and may interfere with the active enzyme and reduce the overall activity of CYP27B1. Alternatively, these variant proteins may also affect pathways that are not related to vitamin D metabolism.

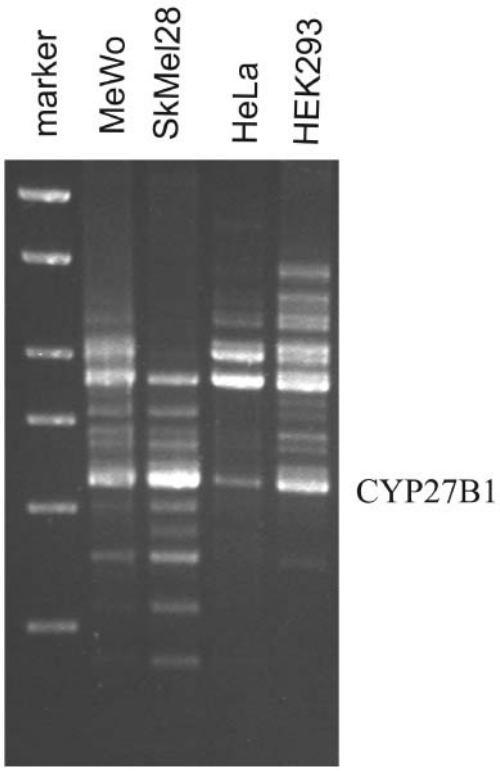

Figure 3. *Nested-touchdown PCR with mRNA from several human cell cultures derived from melanoma (MeWo and SkMel28), cervical carcinoma (HeLa) and embryonic kidney (HEK293). The position of the normal CYP27B1 cDNA sequence is indicated.*

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