Abstract. The LIM-only protein FHL2 (four-and-a-half LIM-domain protein 2) belongs to the FHL protein family of transcriptional cofactors present in various cell lines. FHL2 interacts with a variety of transcription factors known to be involved in tumour development. Furthermore, FHL2 expression is often deregulated in cancer including overexpression and down-regulation in various types of tumours. The function of FHL2 in cancer is particularly intriguing, since it may act as an oncoprotein or as a tumour suppressor in a tissue-dependent fashion. This dual nature of FHL2 is also reflected by the finding that it can function as repressor or activator of transcriptional activity depending on the cell-type. The ability of FHL2 to exert functional diversity lies within its structural composition as a LIM-only protein. LIM-domains are enzymatically inactive protein-to-protein interaction domains, which determine the function of LIM-only proteins as adaptor molecules or scaffolding proteins. By selectively using different LIM-domains for protein-to-protein interactions, FHL2 is capable to interact with a broad spectrum of functionally unrelated proteins, thereby triggering different signalling pathways. In this review, the current knowledge of FHL2 expression in different cancers was summarized and the interaction of FHL2 with transcription factors and other proteins involved in cancer development was examined. Since transcription factors control all fundamental developmental and homeostatic processes, transcriptional cofactors like FHL2 are likely to contribute to human carcinogenesis and are of clinical importance in various forms of cancer.

Cancer is one of the leading causes of death in developed countries and is presently responsible for about 25% of all deaths. Every year 0.5% of the population are diagnosed with cancer (16). It affects people of all ages, but the risk increases dramatically at the age of 60. The most common cancer in adult males is prostate cancer and breast cancer in adult females. The leading lethal type of cancer in both men and women in the US is lung cancer (16). Cancer in young children and adolescents is rare, but can occur. Leukaemia is one of the most common cancers in this group (13). Another type of cancer, rhabdomyosarcoma, arises from muscle tissue and is exclusively found in children (37). The development of cancer can have different causes. Some cancers could be congenital, such as breast cancer, and others are associated with viral infections, such as cervical cancer which correlates to the infection with papilloma-virus (42). Ultimately most cancers develop due to the accumulation of DNA mutations over the years that negatively affect expression of tumour suppressor proteins or positively affect the expression of oncoproteins. Since there is no single cause for the development of a specific type of cancer, no cancers are alike.

The FHL-subfamily belongs to the LIM-only protein family and consists of four LIM-domains and a single zinc finger motif at the amino-terminus. Five members have been described so far, FHL1-4 and ACT (activator of CREM in testis) (6, 27, 28, 43). One intact LIM-domain consists of a double zinc finger structure which mediates protein-to-protein interactions (19, 26). FHL1, FHL2 and FHL3 are mainly expressed in muscle (28, 38), whereas FHL1 and FHL2 can also be found in tissues of different origin (28, 39, 43). The expression of FHL4 and ACT is restricted to testis (27). While FHL proteins can fulfill many different functions depending on the cell-type and the interacting protein, the modulation of transcription factor activity seems to be a common feature for all FHL-proteins.
Structure and Function of FHL2

FHL2 is the best studied member of the FHL-family. It was originally identified by subtractive cloning of normal myoblasts and rhabdomyosarcoma cells (14). This early study demonstrated that the protein is down-regulated in all rhabdomyosarcoma tested compared to healthy muscle tissues. This finding led to its original name DRAL (down-regulated in rhabdomyosarcoma LIM-protein). FHL2 is a protein of 279 amino acids (aa) in length containing four full LIM-domains and a half LIM-domain at the amino-terminus (Figure 1). The FHL2 mRNA encompasses 1416 bp. The corresponding gene was mapped to chromosome 2q12-q13 by FISH (fluorescent in situ hybridisation) analysis. It consists of seven exons, of which only the last four code for the FHL2-polypeptide (4). Due to the differential usage of different LIM-domains, FHL2 is able to interact with many different proteins, i.e., depending on the interaction partner different LIM-domains are involved. The association with FHL2 can be mediated by the full-length protein as described for the interaction with β-catenin (25, 45), by LIM domains ½-2 for FOXO1 (51), by LIM-domains ½-3 for CDC47 (4), by the C-terminal LIM-domains LIM 2-4 for BRCA1 (50) or by the C-terminal LIM-domains 3-4 for NP220 (31). While GAL4-dependent reporter gene assays indicate that the different LIM-domains can have different regulatory effects on their interacting partners, the mode of action requires further elucidation (50).

To date, only very little is known about the regulation of FHL2-expression. Still, there are data indicating that the tumour suppressor protein p53 induces transcription of FHL2 (39). While high levels of FHL2-mRNA were found in primary myoblasts expressing wild-type p53, only low levels of FHL2-mRNA were detectable in rhabdomyosarcoma exhibiting a mutated form of p53. Scholl et al. demonstrated that the transient transfection of wild-type p53 in rhabdomyosarcoma cells reinstated FHL2-expression (39). FHL2 was found to play a role in the regulation of signal transduction, gene expression and cytoskeleton modulation, as well as in cell adhesion, survival and mobility (5, 22, 25, 29, 48). Consistently, FHL2 can be localised in the nucleus, the cytoplasm in association with the cytoskeleton. FHL2 promotes differentiation of muscle precursor cells and acts as a scaffolding protein in mature heart (22, 25).

FHL2-expression in Cancer

Significant differences in the level of FHL2-expression comparing normal and cancer tissues could be observed in many organs (Table I). FHL2 was found to be down-regulated in rhabdomyosarcoma compared to normal myoblasts indicating a role as a tumour suppressor gene in muscle tissue. Concomitantly, overexpression of FHL2 in muscle precursor cells increased myotube formation and, thus, the differentiation of these cells (25). FHL2-expression is also down-regulated in prostate cancer (17). In contrast, overexpression of FHL2 could be detected in ovarian cancer (12), human melanoma (5), lung cancer (43), colon carcinoma (4) or breast cancer (12) compared to normal tissues.

FHL2 in breast cancer. The first indication that FHL2 could play a role in breast cancer development came from a study by Yan et al. showing that FHL2 was expressed in ovarian and breast cancer and was able to interact with the breast cancer susceptibility gene BRCA1 via the carboxy-terminal domain of BRCA1 (50).

Our recent studies revealed that FHL2 was not expressed in normal breast tissue, but malignant tumours showed high expression levels. Interestingly, in the non-malignant “Ductal Carcinoma In Situ” (DCIS), lower levels of FHL2-expression compared to malignant tumours could be detected, also suggesting a role of FHL2 in the development of breast cancer (unpublished data). A typical immunohistochemical staining of FHL2 in breast cancer and normal breast tissue is shown in Figure 2. Further clinicopathological studies demonstrated that FHL2 was overexpressed in breast cancer compared to normal breast tissue (11). Furthermore, the survival rate of breast cancer patients correlated with the level of FHL2-expression. The effect of anticancer treatment on FHL2 expression was also analysed, i.e. treatment with tamoxifen, an antihormone of (β)17-estrogen, partly reversed the negative prognostic impact of high FHL2-expression (11). Interestingly, FHL2 is also able to interact with the estrogen receptor (18). Still, the functional consequences of this interaction remain to be elucidated.

FHL2 in ovarian cancer. Ovarian cancer is an idiopathic cancer. The detailed mechanisms underlying its development are still unknown; however, a few studies have suggested a hereditary disposition. There are also data showing that mutations of BRCA1 and BRCA2 lead to a higher risk of developing ovarian cancer and breast cancer (21). FHL2 is overexpressed in epithelial ovarian cancer compared to normal ovarian tissue (12). Gabriel et al. analysed the localisation of the FHL2 protein in human ovarian cancer cells by immunohistochemical techniques and found that it was mainly localised at the membrane and in the cytoplasm, indicating a role in initial events of signal transduction. In line with this observation, FHL2 interacts with pp125FAK (focal adhesion kinase), a non-receptor tyrosine kinase that is expressed throughout development and in adult tissues (12). pp125FAK is not only involved in integrin-mediated signal transduction, it is also regulated via growth receptors, G-protein receptors and neuropeptides, such as endothelin and bombesin (52). Whether the interaction of FHL2 with FAK contributes to cancer development is still an unanswered question.
FHL2 in prostate cancer. The androgen receptor (AR) plays an important role in prostate cancer progression. Different studies suggest that the expression pattern of the androgen receptor characterises different stages of cancer progression: while in advanced cancer, higher levels of AR compared to normal tissue is expressed, in localised prostate cancer only a low frequency can be detected (41). Recently it was demonstrated that co-regulators of AR were overexpressed in advanced prostate cancers (8). FHL2 was able to interact

Table 1. Expression profile of FHL2 in cancer and normal tissue.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal tissue</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Colon</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lung</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Muscle</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Ovary</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Prostate</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Skin</td>
<td>?</td>
<td>+</td>
</tr>
</tbody>
</table>
with the AR in prostate cancer cell lines and acted as a coactivator of AR-dependent gene expression (30). To elucidate the role of AR transcription in more detail, Kinoshita et al. tested the mRNA expression of different AR cofactors in primary prostate cancers in comparison to normal tissues by real-time PCR (17). These experiments revealed that the expression of FHL2 was decreased 2- to 4-fold in prostate cancer relative to normal tissue (17).

The Interaction of FHL2 with Proteins Involved in Carcinogenesis

The aberrant expression of FHL2 in certain tumours led to the question whether FHL2 can act as an oncprotein or a tumour suppressor in a cell type-specific manner. Most likely, the functional duality of FHL2 in different cancer cells correlates to its potential to act as a transcriptional cofactor. It is known that FHL2 can play a dual role as transcriptional activator or repressor depending on the cell-type in which it is expressed. In muscle cells, FHL2 was identified as an interaction partner of β-catenin and FHL2 was able to repress β-catenin-dependent transcription in a dose-dependent manner (25). On the other hand, FHL2 was identified in hepatoblastoma cells as an activator of β-catenin-dependent transcription (45). Since FHL2 acts mainly as an adaptor protein, it can be postulated that FHL2 has the capability to modulate gene expression by triggering different transcription factors. In fact, many transcription factors interacting with FHL2 are either repressed or activated by FHL2 (Table II).

FHL2 and CDC47 in cell cycle regulation. One of the first studies that revealed FHL2 as a regulator of the cell cycle have demonstrated that FHL2 was able to interact with CDC47 in the nucleus (4). CDC47 is a member of the MCM family (minichromosome maintenance), which plays a role in early stages of chromosomal DNA replication. CDC47 enters the nucleus during mitosis, where it remains until the initiation of DNA replication and is exported back into the cytoplasm at the beginning of S-phase (47). As long as CDC47 localises to the nucleus, it interacts with FHL2 in colorectal adenocarcinoma SW480 and HeLa S3 cells (4). Still, how FHL2 influences the mitotic properties of CDC47 will be subject of future research.

FHL2 and the regulation of steroid hormone-dependent pathways. FHL2 seems also involved in steroid hormone-related pathways. FHL2 is able to interact with the AR in the nucleus of prostate cancer cells (30). Androgen plays an important role in cell-cycle progression. The functional modulation of the AR is achieved by androgen binding leading to the dimerisation, activation and nuclear translocation of the receptor where it regulates gene transcription. Recently it was demonstrated that the AR was also able to mediate MAPK-related signalling. The non-hydrolysable androgen agonist R1881 activated ERK (extracellular signal-related protein kinase) in human breast cancer cells (54) and dihydrotestosterone increased ERK phosphorylation in primary prostate stroma cells (33). Since FHL2 was able to interact directly with the AR, as well as with various partners of the MAPK pathway, such as ERK2 and c-jun (29, 34), it could be concluded that FHL2 might play a role in this pathway. In addition, FHL2 regulates AR-related transcription directly on the DNA level. FHL2 was able to stimulate AR-dependent activity on a naturally occurring promoter indicating another role of FHL2 in prostate cancer progression (30).

In addition, FHL2 interacts with the estrogen receptor (ER), which is usually located in the cytoplasm, but can also be found at the membrane or in the nucleus. The ER is also activated by binding to hormone followed by the formation of homodimers, which directly bind to oestrogen response elements (EREs) on DNA, thereby inducing ER-related transcription. The ER is divided in mainly two subfamilies, the oestrogen receptor alpha (ERα) and the oestrogen receptor beta (ERβ). The ERα can be found in breast cancer cells, ovarian stroma cells, endometrium and the hypothalamus (49), whereas the ERβ is expressed in kidney, brain, bone, heart, prostate and various other tissues (1). Kobayashi et al. revealed that FHL2 interacts with the ERα in the presence of β17-oestradiol, but not in the presence of the anti-estrogens tamoxifen or raloxifen. However, FHL2 did not effect ERα-dependent transcription in reporter gene analysis on an ERE-dependent reporter. Thus, the effect of the interaction of FHL2 and the ER still remains unclear. It is known that oestrogen was able to induce BRCA1

### Table II. Interaction partners of FHL2 involved in carcinogenesis.

<table>
<thead>
<tr>
<th>Interaction partner</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen receptor</td>
<td>Co-activator</td>
<td>Müller et al. 2000 (30)</td>
</tr>
<tr>
<td>AP-1</td>
<td>Co-activator</td>
<td>Morlon and</td>
</tr>
<tr>
<td>BRCA1</td>
<td>unknown</td>
<td>Yan et al. 2003 (50)</td>
</tr>
<tr>
<td>CBP/p300</td>
<td>Co-activator</td>
<td>Labalette et al. 2004 (20)</td>
</tr>
<tr>
<td>CDC47</td>
<td>unknown</td>
<td>Chan et al. 2000 (4)</td>
</tr>
<tr>
<td>E4F1</td>
<td>Co-repressor</td>
<td>Paul et al. 2006 (55)</td>
</tr>
<tr>
<td>ERK2</td>
<td>Co-repressor</td>
<td>Purcell et al. 2004 (34)</td>
</tr>
<tr>
<td>Oestrogen receptor alpha</td>
<td>unknown</td>
<td>Kobayashi et al. 2004 (18)</td>
</tr>
<tr>
<td>FOXO1</td>
<td>Co-repressor</td>
<td>Yang et al. 2005 (51)</td>
</tr>
<tr>
<td>HIPK2</td>
<td>Co-activator</td>
<td>Lee et al. 2006 (23)</td>
</tr>
<tr>
<td>Pp125FAK</td>
<td>Unknown</td>
<td>Gabriel et al. 2004 (12)</td>
</tr>
<tr>
<td>SKI</td>
<td>Co-activator</td>
<td>Chen et al. 2003 (5)</td>
</tr>
<tr>
<td>β1-integrin</td>
<td>unknown</td>
<td>Wixler et al. 2000 (48)</td>
</tr>
<tr>
<td>β-catenin</td>
<td>Co-repressor</td>
<td>Martin et al. 2002 (25)</td>
</tr>
<tr>
<td></td>
<td>Co-activator</td>
<td>Wei et al. 2003 (45)</td>
</tr>
</tbody>
</table>
mRNA-expression by recruiting an AP1/ERα complex to the proximal BRCA1 promoter (15). Since FHL2 can interact with AP1, ERα and BRCA1 (18, 29, 50), it might play a regulative role in this context.

**FHL2 and SKI in human melanoma.** The oncprotein SKI (Sloan-Kettering viral oncogene homologue), which contributes to the progression of melanoma (36), cervical carcinoma (9) and oesophageal squamous cell carcinoma (10), was also found to interact with FHL2, leading to an enhancement of the FHL2-mediated activation of β-catenin-dependent transcription (5). SKI was originally identified as an inhibitor of TGFβ (transforming-growth factor β) in the nucleus by associating with Smad proteins. However, recent studies have indicated that SKI might play a role in cancer progression. Whereas in normal melanocytes and non-invasive melanoma SKI localises exclusively in the nucleus, it was also found in the cytoplasm of primary invasive melanoma. FHL2 might have an apoptotic function in several cell lines including melanoma (5). SKI seems to be able to block FHL2-mediated apoptosis by turning FHL2 into a growth stimulator for mouse melanocytes and human melanoma cells (5).

**FHL2 and p53.** p53 is activated by DNA damage and arrests the cell cycle by inducing p21\(^{Waf1}\), which leads to inhibition of the cyclin D/CDK4/6 complex, thereby giving the cell enough time for necessary repairs. p53 regulates the expression of FHL2 (39). Recently it was also shown that FHL2 was able to regulate p53-dependent transcription through a direct association with HIPK2 (23). HIPK2 has been described as a homeodomain-interacting protein kinase involved in TNF-R1 (tumour necrosis factor receptor type 1)-mediated signalling (24) and therefore plays a role in tumour development. HIPK2 can bind, stabilise and phosphorylate p53, inducing p53 transcriptional activity and apoptotic function (7, 44). Recently a study by Lee et al. revealed that FHL2, p53 and HIPK2 not only form a ternary complex but are also associated with the p21\(^{Waf1}\) promoter. Reporter gene analyses have indicated that HIPK2 enhances the p53-dependent transcriptional activation and that FHL2 is able to increase this process (23). Taken together, these data provide additional evidence for the involvement of FHL2 in cell cycle regulation and cancer development.

**The Role of FHL2 in the Acetylation and Deacetylation of Proteins**

The regulation of gene expression is not restricted to the activity of transcription factors. It can also be modulated on the chromatin level. Chromatin is a complex of DNA and proteins found inside of nuclei. The main components involved in chromatin packaging are histones, which act as spools around which 148-165 bp of DNA winds. By this, the DNA is compacted and 50,000 times shorter than an unpacked molecule. To date, five major histone classes are known in eukaryotes, H1 (also known as the linker histone or H5), H2A, H2B, H3 and H4. H2A, H2B, H3 and H4 are called core histones, which form an octameric nucleosome core particle.

Weintraub and Groudine postulated that because the DNA is condensed as chromatin and not accessible to enzymes, gene expression must involve selective disruption of the folded structure (46), so posttranslational modifications of nucleosomal histones must occur. Nowadays, three methods of chromatin remodelling are known: induction by specialised complexes under the use of ATP (2), replacement of one or more core histones (35, 40) and covalent modifications of histones (53). Of all modifications known, acetylation and deacetylation of the amino-terminal tail of nucleosomal histones are the most widely investigated. Acetylation and deacetylation are mediated by histone acetyltransferases (HAT) and histone deacetylases (HDAC). FHL2 is not only known to interact with histone deacetylases like Sir2uin-1 (SIRT1) and histone deacetylases (HDAC), it can also interact with other regulatory factors of acetylation and deacetylation, such as the Forkhead class box proteins O (FOXO) transcription factors (51). FOXO functions downstream of the PTEN tumour suppressor and directly control the expression of genes involved in apoptosis, cell cycle progression and stress response. FHL2 was identified as an interaction partner of FOXO1 in the nucleus of prostate cancer cells where FHL2 decreases the transcriptional activity of FOXO1. This repression is mediated by the HDAC SIRT1 which is able to deacetylate FOXO1 and inhibits its transcriptional activity. FHL2 enhances this interaction and thereby the deacetylation of FOXO1. This, in turn, leads to repression of FOXO1-dependent transcription and abolishes the repression of FOXO1 on Cyclin D1, indicating that FHL2 is not a classic co-repressor, but functions as an adapter of FOXO1 and SIRT1 (51).

FHL2 was shown to interact with the CBP/p300 acetyltransferase which individually stimulates β-catenin transcriptional activity. CBP/p300 was the first acetyltransferase identified. It acetylates all four core histones and acts as a transcriptional adaptor (32). FHL2, CBP/p300 and β-catenin from a ternary complex in the nucleus, which leads to enhanced β-catenin/TCF-mediated and AR-mediated transcription. Since a number of transcription factors are known to be acetylated by p300/CBP (3), it can be assumed that FHL2 exerts its function as transcriptional cofactor, at least in part, by modulating the activity of HATs and HDACs.
Conclusion

The data summarised in this review strongly indicate an involvement of the transcriptional cofactor FHL2 in the progression or suppression of cancer growth. Nevertheless, data showing a direct contribution of FHL2 to cancer growth in the literature are still missing, indicating the need for further studies to evaluate this aspect in more detail. For instance, a stable knock-down of FHL2 in cancer cells by using siRNA-based techniques would help to study the contribution of FHL2 to important malignant features of tumour cells, such as contact inhibition, the ability to form colonies in soft agar or anchorage-independent growth. Furthermore, FHL2-depleted cancer cells could be used for Xenograft experiments in nude mice to analyse the impact of FHL2 on cancer growth and invasion in vivo. If future experiments would identify FHL2 as a cell-specific oncogene or tumour-suppressor, FHL2 could become an attractive target for therapeutical interventions. Understanding the mechanisms behind the potential dual function of FHL2 as tumour suppressor or oncoprotein will especially help to understand how transcriptional cofactors, the most distal elements of signalling pathways, contribute to human carcinogenesis.

Acknowledgements

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References


