Diverse LEF/TCF Expression in Human Colorectal Cancer Correlates with Altered Wnt-Regulated Transcriptome in a Meta-Analysis of Patient Biopsies

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Abstract: Aberrantly activated Wnt signaling causes cellular transformation that can lead to human colorectal cancer. Wnt signaling is mediated by Lymphoid Enhancer Factor/T-Cell Factor (LEF/TCF) DNA-binding factors. Here we investigate whether altered LEF/TCF expression is conserved in human colorectal tumor sample and may potentially be correlated with indicators of cancer progression. We carried out a meta-analysis of carefully selected publicly available gene expression data sets with paired tumor biopsy and adjacent matched normal tissues from colorectal cancer patients. Our meta-analysis confirms that among the four human LEF/TCF genes, LEF1 and TCF7 are preferentially expressed in tumor biopsies, while TCF7L2 and TCF7L1 in normal control tissue. We also confirm positive correlation of LEF1 and TCF7 expression with hallmarks of active Wnt signaling (i.e., AXIN2 and LGR5). We are able to correlate differential LEF/TCF gene expression with distinct transcriptomes associated with cell adhesion, extracellular matrix organization, and Wnt receptor feedback regulation. We demonstrate here in human colorectal tumor sample correlation of altered LEF/TCF gene expression with quantitatively and qualitatively different transcriptomes, suggesting LEF/TCF-specific transcriptional regulation of Wnt target genes relevant for cancer progression and survival. This bioinformatics analysis provides a foundation for future more detailed, functional, and molecular analyses aimed at dissecting such functional differences.

Keywords: Wnt; colorectal cancer; TCF; LEF; transcriptome

1. Introduction

Wnt signaling functions in normal development and stem-cell-mediated homeostasis; and also, in the etiology of disease, such as cancer [1,2]. The best understood Wnt signaling mechanism is a nuclear β-catenin-mediated signal transduction pathway regulating transcriptional gene expression [3]. Wnt pathway-promoted nuclear β-catenin proteins regulate transcription indirectly by binding to and altering multi-protein complexes associated with sequence-specific DNA binding factors, predominantly of the Lymphoid Enhancer Factor/T-Cell Factor (LEF/TCF) protein family [4,5]. Humans have four genes encoding LEF/TCF proteins, similar to other mammals and most vertebrates [6,7]. With little or without nuclear β-catenin protein, LEF/TCF proteins mostly mediate...
transcriptional repression of Wnt-target genes. Moreover, with increased nuclear β-catenin levels, LEF/TCF proteins generally mediate transcriptional activation [8].

Different LEF/TCF genes are expressed in different tissues and at different stages, and together with alternative splicing and alternative promoter use this results in a rich variety of differentially expressed LEF/TCF protein isoforms [7,9,10]. There is considerable functional redundancy between different LEF/TCF proteins, yet also emerging evidence for quantitative and even qualitative differences. Quantitative difference refers here to different LEF/TCF protein isoforms being more or less effective at mediating transcriptional repression with little or without nuclear β-catenin protein; versus transcriptional activation with increased nuclear β-catenin levels [4,6,11,12]. Qualitative difference refers to the possibility of different LEF/TCF protein isoforms binding to different cis-regulatory target DNA sequences and thereby regulating different Wnt target genes [13,14].

Wnt signaling is particularly relevant in colorectal cancer, since the vast majority of colorectal cancers harbor Wnt/β-catenin pathway-activating mutations [15], which contrasts with a normal benevolent role in regulating stem-cell-mediated maintenance of intestinal and colorectal epithelial tissue [16,17]. Roles for LEF/TCF proteins have been studied in tissue-culture and animal models of intestinal stem cells and colorectal cancer, which suggested roles for TCF7L2 and TCF7 in normal colorectal tissue, where LEF1 and TCF7L1 are silent, but strong expression in colorectal tumor of LEF1 [9,18–21] and TCF7 [22,23].

Here, we aimed to assess whether predictions about Wnt signaling in colorectal cancer from tissue-culture and animal model systems could be confirmed by a meta-analysis of compatible transcriptomics studies of paired normal and tumor biopsy samples from human patient, and whether specific LEF/TCF gene-correlated transcriptomes imply LEF/TCF gene-specific function in normal or tumor tissue.

Our meta-analysis of transcriptomics studies provides a clear picture of altered LEF/TCF gene expression, confirming TCF7L2 in normal and LEF1 expression in tumor tissue, but also higher than expected TCF7L1 in normal and relatively higher TCF7 expression in tumor tissue. Specific LEF/TCF gene expression is indeed correlated with differences in the overall transcriptome, with some of these differences suggestive of relevance for tumor progression.

2. Materials and Methods

Publically available data were searched within the databases Gene Expression Omnibus (GEO) and ArrayExpress using ‘colon cancer’ or ‘colorectal cancer’ as search words. From this list only studies that satisfied the following conditions were selected: a) samples from human biopsies (i.e. no rodent models or cell line data), b) paired samples from tumor with nearby normal tissue, c) a sample size of at least four patients in order to conduct meaningful statistical analyses. The selected studies with Genomic Spatial Event (GSE) database accession numbers are listed in Table 1. As a first exploratory analysis step a principal component analysis (PCA) based on scaled and centered variables was conducted using the `prcomp` function within R. The score plots of the first two principal components were studied to check for the separation of tumor and normal samples.

Meta-analysis of differential expression between tumor and normal for the eight selected genes of interest (Figure 1) was conducted using the R-package metafor [24]. The standardised mean difference (SMD) was chosen as a measure of differential gene expression here and a ‘random effects’ model was used for weighting of the studies. The random effects model is used to address the heterogeneity between studies caused by differences in study designs, study population and also by the different gene expression platforms used.

The correlation plots (Figure 2) were produced within the R-package corrplot [25]. For the meta-analysis of correlation differences between normal and tumor for each of the eight selected genes of interest we first selected 18,150 genes that were included in at least two of the studies. For each of the eight genes, we then calculated correlations within tumor and normal samples with each of the 18,150 other genes. The differences between tumor and normal correlation for each of the 8 × 18,150 = 145,200 combinations where averaged across the studies and tested against the null hypothesis of no correlation by a one-sample t-test, which corresponds to a random effect
meta-analysis, where within study variation is assumed to be negligible compared to between study variation.

The organized expression data (Supplementary Table S1B–I) were filtered for standard deviation less than 0.2 where correlation coefficients association with a specific gene were ranked in all normal or all tumor samples (e.g., Supplementary Table S2), and where mean difference between them was independently calculated for p-value less than 0.05 (e.g., Table 2). The top 10 genes were presented in a ranked list (Tables 2–4; Supplementary Tables S2–4), and the top 100 list was used for gene ontology analysis, excluding the target gene itself (i.e. AXIN2 in the AXIN2 list, the specific LEF/TCF gene in the specific LEF/TCF gene list etc.). The individual LEF/TCF correlation values were compared with each other (Supplementary Figure S1J), and with AXIN2 (Supplementary Figure S1K). Gene ontology analysis was carried out on GOrrilla (http://cbl-gorilla.cs.technion.ac.il) selecting the ‘Two unranked lists of genes’ setting comparing the first 100 genes of the ranked gene lists (see above) with the full 18,150 background list of all genes in the analyzed data. The top 5 gene ontology terms were listed together with order of magnitude or their p-value if below 0.001.

### Table 1. Data Sets Mined in this Investigation.

| Name           | Description                                                                 | Reference |
|----------------|-----------------------------------------------------------------------------|-----------|
| GSE10950       | 24 colon normal and tumor pairs (48 arrays)                                  | [26]      |
| GSE20842       | Paired samples of tumor and mucosa from a total of 65 patients (130 arrays). 30 of the patients carried mutated KRAS1. this data set was divided into two different studies: one with mutated KRAS (named «mutated») and one without (named «wild type»). | [27]      |
| GSE25070       | 26 pairs of fresh frozen colorectal tumor and matched adjacent non-tumor tissue samples (52 arrays). | [28]      |
| GSE44076       | 98 colorectal cancer patients and their pairs (196 arrays).                  | [29]      |
| GSE46622       | 4 colorectal cancer patients and their pairs (8 arrays).                     | [30]      |
| GSE50760       | 18 patients, colon and tumor paired (36 arrays).                            | [31]      |

### 3. Results

#### 3.1. Selection of Transcriptomics Data Sets and Quality Control

We carefully reviewed publicly available transcriptomics datasets of human colorectal cancer samples. We focused on the traditional and longest existing gene expression databases GEO and ArrayExpress. We selected published studies with a) sufficient numbers of samples for the intended statistical meta-analysis and b) paired samples from tumor with nearby normal tissue (Table 1). A similar review of available human RNA-seq datasets at the time did not identify studies with sufficient numbers of samples to justify a meaningful meta-analysis. Our initial selection of studies therefore contained mainly microarray studies as these form the majority of entries in those databases and also because the few RNAseq studies we obtained had either too small a sample size or other quality issues. We also note that a joint meta-analysis combining microarray and RNAseq data would be quite challenging as the nature of the data (continuous measurements versus normalized counts) and the corresponding analysis techniques (linear models versus generalized linear models) are fundamentally different.

Our hypothesis predicts that the transcriptome in tumor tissue would be sufficiently distinct from the one in control normal tissue as a prerequisite for our intended meta-analysis. A principal component analysis of the selected datasets (Supplementary Figure S1) therefore served as an additional quality control confirming separation of the transcriptome between tumor sample and normal control in all selected individual studies. The data from Kim et al. [31] showed a somewhat less distinct separation of tumor versus normal transcriptome, but a clear enough difference to retain this study in our meta-analysis. Another study, GSE46905 [32], which we had originally considered, was not taken forward because we could not find sufficiently clear separation between tumor and normal transcriptome (Supplementary Figure S2).
3.2. Transcriptomics Expression of Eight Selected Genes

We first tested our hypothesis about differential expression of LEF/TCF genes between tumor and normal tissue with forest plots (Figure 1) of all four LEF/TCF genes (TCF7, LEF1, TCF7L1, TCF7L2). We chose to monitor additionally the expression of AXIN2, DKK1, FZD7, and LGR5. AXIN2 is a direct Wnt target gene [33,34] used here as a reliable indicator of intracellular Wnt/β-catenin pathway activity, and because increased expression has been reported in colorectal tumor tissue [35]. DKK1 is a direct Wnt target gene [36] with increased expression in many cancers [37], however, here DKK1 was chosen particularly because its expression had previously been reported to be reduced in colorectal tumor [38]. FZD7 is also a Wnt target gene [39], relevant in colorectal cancer [40] and normal intestinal epithelium [41]. LGR5 is a Wnt target gene, which is the marker gene for normal adult intestinal stem cells [17], and for particularly aggressive colorectal cancer stem cells [16,22,23,42].

Our meta-analysis reveals that AXIN2 (Figure 1E) is consistently expressed at a higher level in tumor relative to normal tissue, and so is LGR5 (Figure 1H), indicating as expected increased Wnt/β-catenin signaling activity [35] and increased stem cell identity of tumor tissue [42]. Importantly, among the LEF/TCF genes, our meta-analysis also corroborates a switch from relatively higher TCF7L2 (Figure 1D) and TCF7L1 (Figure 1C) expression in normal control to relatively higher TCF7 (Figure 1A) and LEF1 (Figure 1B) expression in tumor tissue. Our meta-analysis did not highlight any dramatic changes in gene expression for the FZD7 and DKK1 genes between normal and tumor tissue.

3.3. Correlation of Expression between Eight Selected Genes

We next analyzed correlations in gene expression between those eight genes, positive or negative, initially in individual matrix plots (Figure 2). As expected, there is in general a positive correlation between AXIN2 expression and LGR5 expression (stem cell identity marker). There appears also generally a positive correlation between TCF7 and LEF1 expression and between those and LGR5 expression, consistent with previous findings in mouse models [22,23]. If these correlations were linked to increased Wnt/β-catenin signaling activity, then we would also expect a positive correlation between TCF7 and LEF1 expression and AXIN2 expression, which indeed is consistent with the data. Interestingly, AXIN2 expression, which indicates Wnt/β-catenin pathway activity, is negatively correlated with TCF7L1 expression. Comparing individual blots also suggests generally less strong correlation in tumor tissue.

We combined the transcriptomics data from these different studies in a meta-analysis, which provided an even clearer picture (Figure 3). However, we only carried out this meta-analysis with data sets from five of the six selected studies (having removed the data set with only a few patients and missing LEF1 values, GSE46622 [30]). This meta-analysis allowed us to tease out more clearly differences between tumor and normal tissue; with correlations between transcripts among these eight genes being mostly much stronger and clearer in normal control tissue (Figure 3A) and generally much weaker in tumor samples (Figure 3B,D). Positive correlations linking TCF7 with LEF1 and with LGR5 in normal tissue are reduced in tumor tissue, and the negative correlation between AXIN2 and TCF7L1 in normal tissue is also reduced in tumor tissue. The exception to this rule is a strengthened correlation between AXIN2 and TCF7 expression in tumor tissue. Remarkably, our analysis also reveals that FZD7 expression is strongly positively correlated with TCF7L1 expression. Even more remarkably, this correlation is absent or much reduced in tumor tissue, and in the one study that has relevant information on kRAS mutant status in samples [27], it suggests that this correlation between TCF7L1 and FZD7 is particularly strong in KRAS-mutant normal tissue (Figure 2C), and is strongly reduced in KRAS mutant tumor (Figure 2D).
Figure 1. Forest plots of gene expression of all four LEF/TCF genes (A): TCF7, (B): LEF1, (C): TCF7L1, (D): TCF7L2, and (E): AXIN2, (F): DICKKOPF-1 (DKK1), (G): FZD7, and (H): LGR5, from six selected studies. Columns from left to right indicate: accession no. of study (with GSE20842 [27] separated between kras-positive and kras-mutant samples); number of patients in individual studies; horizontal segments indicate the standardized mean difference between tumor and normal, and their confidence interval, with the size of the square dot being proportional with the weight of the study in the meta-analysis using a ‘random effects’ model. The corresponding values are written in the column on the right: weight of the individual study in percent as part of the meta-analysis; standardized mean difference; and in square brackets confidence interval. The red polygon in the bottom of each plot shows the summary estimate based on the random-effect model. Values to the left of the midline indicated higher expression in the control relative to the tumor sample, e.g., see AXIN2 and LGR5. Individual studies with small sample size (i.e., few patients) as expected often have larger confidence intervals (therefore less reliability, e.g., see TCF7 and LEF1 data for GSE46622 study), but in the meta-analysis (in red) much tighter confidence intervals (therefore higher reliability). Note that, among the four LEF/TCF genes, TCF7, LEF1, are expressed higher, while TCF7L1, TCF7L2 lower in tumor tissue.
Figure 2. Correlation plot matrix of relative gene expression between eight selected genes in six selected studies (A–N), with normal control (A, C, E, G, I, K, M) separated from tumor sample (B, D, F, H, J, L, N), and additionally for the GSE20842 [27] between kras-mutant (“mut”) samples (C, D) and kras-positive (“wild” as in wildtype) samples (E, F). Blue dots indicate positive and red dots negative correlation. The size of the circle and the intensity of the color is proportional to the correlation coefficient; therefore, as an internal control, expected diagonal series of large blue dots where expression of genes is compared to the expression of the same gene). Missing values in GSE46622 [30] is due to low value data for LEF1 in this study. Note positive correlation between AXIN2 expression and LGR5, TCF7 and LEF1 expression, yet negative correlation with TCF7L1 expression, while TCF7L1 and FZD7 expression are positively correlated, though clearly much more so in normal control tissue than in tumor. In contrast, the correlation between AXIN2 and TCF7 expression is clearly more robust in tumor compared to normal tissue. Interestingly, the unearthed correlation between TCF7L1 and FZD7 expression appears to be dependent on wild-type kRAS in the tumor (compare D with F, yet not in normal control C).
3.4. Correlation between Eight Selected Genes and the Rest of the Transcriptome

We extended the analysis for correlations in gene expression between each of those eight genes with the whole rest of the transcriptome. We ranked the TCF7 gene expression-correlated whole transcriptome expression (positively and negatively correlated) separately in normal tissue and in tumor tissue (Supplementary Table S2A), and then also independently ranked and analyzed the greatest transcript correlation differences between tumor and normal tissue (Table 2). In a gene ontology analysis, we searched for the suggested association of correlated gene expression with biomedical processes. We then repeated this independently for the other LEF/TCF gene expression-correlated transcriptomes, and the AXIN2-, DKK1-, FZD7-, and LGR5-correlated transcriptomes (Table 2, Supplementary Table S1B-I, Supplementary Table S2).

In normal tissue, both TCF7 and LEF1 expression is positively correlated with gene expression associated with the immune system. This correlation with immune system-associated transcripts is more generally lost in tumor tissue. In normal tissue TCF7L1 expression is positively, and AXIN2 expression negatively correlated with gene expression associated with cell adhesion. While in tumor tissue TCF7L1, and even more so LEF1 gene expression is correlated with transcripts associated with the extracellular matrix (ECM); and expression of AXIN2 with TCF7 is correlated with regulation of Wnt signaling (particularly Wnt receptor catabolic processes), with this correlation being stronger in tumor than normal tissue. However, we do not find any specific correlation with cyclin D1 (CCND1) gene expression [43,44], and BMP4 expression [45] is only specifically correlated with DKK1.
| Table 2. Correlated Transcriptome. |
|----------------------------------|

**A: Largest Difference in the TCF7-Correlated Transcriptome between Tumor and Normal**

| Ranked Gene List Top 10 | More in Tumor          | Less in Tumor          |
|-------------------------|------------------------|------------------------|
| NECAB3, TCFL5, ZNRF3, IFT52, SLC2A12, SKP1, DYNLRB1, MBNL2, SSX2IF, CAMLG | WDFY4, TBC1D1O, TRAF3IP3, IKZF1, RASAL3, SP140, LT, ARHGAP9, CD37, SASH3 |

Associated Gene Ontology Top 5:
- intracellular transport involved in cilium assembly (10^-6)
- intracellular transport (10^-3)
- protein-containing complex localization (10^-9)
- Wnt signaling pathway involved in cell-cell signaling (10^-9)

**B: Largest Difference in the LEF1-correlated transcriptome between Tumor and Normal**

| Ranked Gene List Top 10 | More in Tumor          | Less in Tumor          |
|-------------------------|------------------------|------------------------|
| SPRR2F, PDGFC, CAB39L, GRP, SOX11, COL12A1, ISM1, GLRB, DKK3, OLFML3 | FAIM3, PVRIG, UGT1A10, CD79B, BANK1, PTPRCAP, BLK, NAPSB, FCRLA, CD19 |

Associated Gene Ontology Top 5:
- extracellular matrix organization (10^-13)
- cell adhesion (10^-8)
- biological adhesion (10^-7)
- extracellular structure organization (10^-12)
- anatomical structure development (10^-9)

**C: Largest Difference in the TCF7L1-correlated transcriptome between Tumor and Normal**

| Ranked Gene List Top 10 | More in Tumor          | Less in Tumor          |
|-------------------------|------------------------|------------------------|
| DCBLD1, HMHA1, ARHGAP17, SH3KBP1, LNX, TMIG1, SLC2A4A6, JAG1, LRCH4, VAMP8 | SSX2IF, SAE1, RHEB, CKMT2, CCNI, NAP1L1, BAG2, ZFAND1, PHGDH, RP55 |

Associated Gene Ontology Top 5:
- angiogenesis (10^-9)
- anatomical structure formation involved in morphogenesis (10^-9)
- positive regulation of neuron death (10^-9)
- blood vessel endothelial cell proliferation involved in sprouting angiogenesis (10^-9)
- regulation of mast cell activation involved in immune response (10^-9)

**D: Largest Difference in the TCF7L2-correlated transcriptome between Tumor and Normal**

| Ranked Gene List Top 10 | More in Tumor          | Less in Tumor          |
|-------------------------|------------------------|------------------------|
| C3orf70, CDC42EP2, DNAJC24, FLJ25758, CDRT15, HIG2, C1T, GNG3, C3orf77, TSPAN5 | ACP5, AOAH, SCAND3, PPP1R14C, KIAA0247, DNAH14, NECAP2, CHMP1B, C3orf20, CTSD |

Associated Gene Ontology Top 5:
- optic nerve development (10^-4)

**Gene Ontology**

**Top 5:**
- intracellular transport involved in cilium assembly (10^-6)
- intracellular transport (10^-3)
- protein-containing complex localization (10^-9)
- Wnt signaling pathway involved in cell-cell signaling (10^-9)
- extracellular matrix organization (10^-13)

**Top 5:**
- extracellular matrix organization (10^-13)
- cell adhesion (10^-8)
- biological adhesion (10^-7)
- extracellular structure organization (10^-12)
- anatomical structure development (10^-9)

**Top 5:**
- angiogenesis (10^-9)
- anatomical structure formation involved in morphogenesis (10^-9)
- positive regulation of neuron death (10^-9)
- blood vessel endothelial cell proliferation involved in sprouting angiogenesis (10^-9)
- regulation of mast cell activation involved in immune response (10^-9)

**Top 5:**
- optic nerve development (10^-4)

**Gene Ontology**

**Top 5:**
- lymphocyte activation (10^-27)
- immune system process (10^-23)
- regulation of immune system process (10^-20)
- leukocyte activation (10^-20)
- positive regulation of immune system process (10^-19)

**Top 5:**
- immune system process (10^-21)
- immune response (10^-18)
- regulation of immune system process (10^-18)
- regulation of lymphocyte activation (10^-15)
- regulation of leukocyte activation (10^-14)

**Top 5:**
- organic substance metabolic process (10^-9)
- cellular metabolic process (10^-9)
- metabolic process (10^-9)
- primary metabolic process (10^-9)
- nucleotide-excision repair, DNA damage recognition (10^-9)
### E: Largest Difference in the AXIN2-correlated transcriptome between Tumor and Normal

**Ranked Gene List Top 10**
- NKD1, CAB39L, APCDD1, NRXN3, PRSS23, LY6G6D, CCDC46, PPP2R2C, HABP4, CKMT2

**Associated Gene Ontology Top 5:**
1. regulation of cellular localization ($10^{-4}$)
2. cellular response to gamma radiation ($10^{-4}$)
3. Wnt signaling pathway ($10^{-4}$)
4. regulation of cell communication ($10^{-4}$)
5. regulation of vascular endothelial growth factor receptor signaling pathway ($10^{-4}$)

**E: Top 5:**
4. negative regulation of eosinophil activation ($10^{-5}$)
   negative regulation of MyD88-dependent toll-like receptor signaling pathway ($10^{-5}$)

### F: Largest Difference in the DKK1-correlated transcriptome between Tumor and Normal

**Ranked Gene List Top 10**
- MIZF, BMP4, SLITRK6, OR7E91P, MGC34774, PANK3, PAQR8, ATAD4, GDA, GPR110

**Associated Gene Ontology Top 5:**
1. lipid catabolic process ($10^{-5}$)
2. cellular lipid catabolic process ($10^{-5}$)
3. molting cycle process ($10^{-5}$)
4. hair cycle process ($10^{-5}$)
5. Golgi reassembly ($10^{-4}$)

**F: Top 5:**
1. positive regulation of ATPase activity ($10^{-4}$)

### G: Largest Difference in the FZD7-correlated transcriptome between Tumor and Normal

**Ranked Gene List Top 10**
- RNFT1, DERL1, FAM49B, FAM91A1, CA13, SLC7A8, ARFGEF1, HHTATIP2, B3GNT2, NUP62CL

**Associated Gene Ontology Top 5:**
1. Golgi reassembly ($10^{-4}$)
2. regulation of developmental process ($10^{-5}$)
3. extracellular matrix organization ($10^{-5}$)
4. extracellular structure organization ($10^{-4}$)
5. regulation of systemic arterial blood pressure by hormone ($10^{-4}$)

**G: Top 5:**
1. anatomical structure development ($10^{-4}$)
2. developmental process ($10^{-4}$)
3. regulation of heart rate by chemical signal ($10^{-4}$)
4. proximal/distal pattern formation ($10^{-4}$)
5. negative regulation of protein polymerization ($10^{-4}$)

### H: Largest Difference in the LGR5-correlated transcriptome between Tumor and Normal

**Ranked Gene List Top 10**
- ZAK, ISM2, SRPK3, SATB1, GRP, ACOT9, ZCCHC12, KLHL23, HEY2, FAH2D8

**Associated Gene Ontology Top 5:**
1. regulation of systemic arterial blood pressure by atrial natriuretic peptide ($10^{-8}$)
2. regulation of developmental process ($10^{-8}$)
3. extracellular matrix organization ($10^{-8}$)
4. extracellular structure organization ($10^{-8}$)
5. regulation of systemic arterial blood pressure by hormone ($10^{-8}$)

**H: Top 5:**
1. B cell receptor signaling pathway ($10^{-6}$)
2. adaptive immune response ($10^{-6}$)
3. immune system process ($10^{-6}$)
4. antigen receptor-mediated signaling pathway ($10^{-6}$)
5. immune response ($10^{-6}$)
Transcriptome correlated to TCF7 (A), LEF1 (B), TCF7L1 (C), TCF7L2 (D), AXIN2 (E), DKK1 (F), FZD7 (G), and LGR5 (H), largest differences between normal and tumor tissue. (ranked lists of top 10 genes of mean differences \(p \leq 0.05\) with top 5 Gene Ontology terms listed if \(p\)-value <10\(^{-5}\), shaded if 10\(^{-4} < 10^{-5}\), in normal font if 10\(^{-5} < 10^{-6}\), and in bold if <10\(^{-10}\), see also Suppl. Table S1B–I and Supplementary Table S2).
3.5. Comparison of Lef/Tcf-Correlated Transcriptomes

Subsequently, we explicitly focused on comparing the different LEF/TCF gene-correlated transcriptomes with each other (Table 3, Supplementary Tables S1J and S3). A compound analysis of differences between all LEF/TCF gene-correlated transcriptomes (Supplementary Table S3) reveals that, overall, differences between TCF7L1- and TCF7L2-correlation dominate in normal tissue, while in tumor tissue, though they are still prominent, differences in particular with TCF7-correlation and also with LEF1-correlation become more noticeable. These overall differences can be associated with extracellular matrix, angiogenesis, and cell adhesion. However, clearly this compound analysis by itself does not reflect any meaningful biological or clinical situation and this gene ontology association here only serves to guide more specific analyses (Table 3).

Detailed pairwise analysis of these prominent differences between TCF7L1- and TCF7L2-correlation in normal tissue suggests a stronger association of TCF7L1-correlated transcripts with the extracellular matrix, and a stronger association of TCF7L2-correlated transcripts with cell junctions and cell adhesion; but interestingly, in tumor tissue TCF7L1 expression is more strongly correlated with transcripts associated with cell adhesion; and also with angiogenesis. Consistent with the single LEF/TCF gene correlation analysis above, any comparison between either TCF7 or LEF1, on the one hand and with either TCF7L1 or TCF7L2, on the other, highlights the higher correlation of expression of TCF7 and LEF1 with a transcriptome associated with the immune system. Remarkably, these pairwise comparisons also reveal that any link to extracellular matrix (ECM) generally excludes TCF7 and TCF7L2, but generally includes both TCF7L1 and LEF1; TCF7L1 more in normal tissue, and LEF1 more in tumor. Comparison between these two, i.e. between TCF7L1- and LEF1-correlated transcription, also reveals a potential correlation of LEF1 expression with double strand break DNA repair in tumor. Pairwise comparison with TCF7, suggests LEF1 in tumor may also be correlated with regulation of cell migration. The same comparison suggests a stronger correlation for TCF7 in normal tissue to transcripts associated with sensory perception, which seems difficult to explain.

3.6. Comparison between AXIN2- and LEF/TCF-Correlated Transcriptomes

Since LEF/TCF proteins are known to function generally as nuclear effectors of WNT/β-catenin signal transduction [3,4,6], we compared individual LEF/TCF gene expression-correlated transcriptomes with the AXIN2 expression-correlated transcriptome (Table 4; Supplementary Table S1K and S4), employing AXIN2 expression again as an indicator of WNT/β-catenin pathway activity. A compound analysis of overall differences between the AXIN2- and all LEF/TCF gene expression-correlated transcriptomes (Supplementary Table S4) reveals that most differences are with the TCFL1-correlated transcriptome, particularly in normal tissue, suggesting a link with cell adhesion.

The direct comparison between the AXIN2- and all TCF7L1 gene expression-correlated transcriptomes confirms the higher correlation of TCF7L1 expression with cell adhesion-associated transcripts, not just in normal tissue, but also in tumor tissue. Remarkably, LEF1 shares with TCF7L1 this stronger link to cell adhesion in tumor tissue, and also an association in normal tissue with muscle, which seems difficult to explain, but could somehow be linked to shared molecular machinery functioning in cell migration. The AXIN2-correlated transcriptome in tumor tissue trumps however, in its association with transcripts indicating regulation of Wnt signaling, particularly Wnt receptor catabolic processes, and contrasting with all LEF/TCF-correlated transcriptomes, apart from, interestingly, that of TCF7.
Table 3. Comparison of LEF/TCF-Correlated Transcriptomes.

|                | In Normal Tissue | In Tumor Tissue |
|----------------|------------------|-----------------|
|                | Ranked Gene List | Ranked Gene List |                |
|                | Top 10           | Top 10          |                |
| A: Differences between TCF7 and LEF1 Correlated Transcriptomes | | |
| Higher TCF7 Correlation | FLJ46257, AADACL4, OR4D9, OR8U9, OR1S1, KRTAP6-1, TRIM6-TRIM34, OR5H15, OR4M2, OSTN | HMHA1, PTPN7, SPI140, PTPRCAP, CD6, FAIM3, DENND2D, SYK, LCK, DENND1C | IFNA1, AADACL1, LEF1, SCYL1BPI, EIF4EBP3, POLR2J3, FAM18B, WDR40A, NME1-NME2, RTCD1, OR51A2 |
|                | 1. detection of chemical stimulus involved in sensory perception of smell (10^-30) | 1. regulation of immune system process (10^-29) | 1. flavone metabolic process (10^-4) |
|                | 2. detection of chemical stimulus involved in sensory perception (10^-30) | 2. regulation of lymphocyte activation (10^-30) | 2. flavonoid glucuronidation (10^-5) |
|                | 3. detection of stimulus involved in sensory perception (10^-27) | 3. regulation of cell activation (10^-30) | 3. extracellular matrix organization (10^-39) |
|                | 4. detection of stimulus (10^-29) | 4. regulation of leukocyte activation (10^-29) | 3. anatomical structure morphogenesis (10^-40) |
|                | 5. G protein-coupled receptor signaling pathway (10^-23) | 5. immune system process (10^-13) | 4. regulation of cell migration (10^-4) |
|                |                  |                  | 5. regulation of cell motility (10^-7) |
|                | GBL, C2orf24, NECAB3, EIF6, MGAT4B, ACOT8, ACSF3, TMUB1, SLC35C2, OR2J3 | C20orf118, VDAC1, EIF6, ETV4, MST4, GPR89A, TRAP1, UBAC2, EP4HA1, ARPC1B | FAM127C, JAZF1, TSPAN2, ZEB1, CAP2, FBXL2, MEIS1, CY51, AGTR1, EPHB1 |
|                | 1. positive regulation of mitochondrial translation (10^-7) | 1. regulation of immune system process (10^-29) | 1. muscle system process (10^-30) |
|                | 2. sulfur compound metabolic process (10^-4) | 2. regulation of lymphocyte activation (10^-30) | 2. muscle contraction (10^-5) |
|                | 3. glycerol metabolic process (10^-3) | 3. regulation of cell activation (10^-29) | 3. actin-mediated cell contraction (10^-4) |
|                | 4. positive regulation of cellular amide metabolic process (10^-2) | 4. regulation of leukocyte activation (10^-29) | 4. system process (10^-7) |
|                | 5. alditol metabolic process (10^-4) | 5. immune system process (10^-13) | 5. regulation of muscle contraction (10^-9) |

B: Differences between TCF7- and TCF7L1-correlated transcriptomes

|                | In Normal Tissue | In Tumor Tissue |
|----------------|------------------|-----------------|
|                | Ranked Gene List | Ranked Gene List |                |
|                | Top 10           | Top 10          |                |
|                |                  |                  |                |
|                | IFNA1, AADACL1, LEF1, SCYL1BPI, EIF4EBP3, POLR2J3, FAM18B, WDR40A, NME1-NME2, RTCD1, OR51A2 | FAM127C, JAZF1, TSPAN2, ZEB1, CAP2, FBXL2, MEIS1, CY51, AGTR1, EPHB1 |  |
|                | 1. flavone metabolic process (10^-4) | 1. muscle system process (10^-30) |  |
|                | 2. flavonoid glucuronidation (10^-5) | 2. muscle contraction (10^-5) |  |
|                |                  | 3. extracellular matrix organization (10^-39) |  |
|                |                  | 3. anatomical structure morphogenesis (10^-40) |  |
|                |                  | 4. regulation of cell migration (10^-4) |  |
|                |                  | 5. regulation of cell motility (10^-7) |  |
### C: Differences between TCF7- and TCF7L2-correlated transcriptomes

#### In Normal Tissue

**Ranked Gene List Top 10**

1. **FAM113B, GRAP, LYL1, HVCN1, CXCR5, FAM65B, FAM129C, LIMD2, KR11, CCL21**
2. lymphocyte activation (10^9)
3. regulation of T cell activation (10^9)
4. B cell activation (10^7)
5. leukocyte activation (10^7)
6. positive regulation of double-strand break repair via homologous recombination (10^8)

#### In Tumor Tissue

**Ranked Gene List Top 10**

1. **TMEM198, PRPF6, GTF3C5, EIF3G, DSN1, SLC35C2, NECAB3, EIF6, RELL2, SNHG11**
2. heterocycle metabolic process (10^-4)
3. cellular nitrogen compound metabolic process (10^-4)
4. nucleobase-containing compound metabolic process (10^-4)
5. snRNA modification (10^-4)
6. viral translational termination-reinitiation (10^-4)

### D: Differences between LEF1- and TCF7L1-correlated transcriptomes

#### In Normal Tissue

**Ranked Gene List Top 10**

1. **HMHA1, DENND2D, UGT1A10, SSH2, BCL11B, SP140, CD6, STX19, ZNF101, EZH2**
2. regulation of immune system process (10^-9)
3. immune system process (10^-8)
4. regulation of cell activation (10^-10)
5. regulation of lymphocyte activation (10^-10)
6. regulation of cytokine production (10^-9)

#### In Tumor Tissue

**Ranked Gene List Top 10**

1. **VDAC1, NCAPG2, NOMO3, EZH2, GPR89A, FAM72A, NOP16, DNAJC2, USP6NL, C20orf118**
2. nucleic acid metabolic process (10^-9)
3. DNA metabolic process (10^-8)
4. double-strand break repair via homologous recombination (10^6)
5. positive regulation of biological process (10^-6)
6. negative regulation of cytoplasmic translational elongation (10^-6)

### E: Differences between LEF1- and TCF7L2-correlated transcriptomes

#### In Normal Tissue

**Ranked Gene List Top 10**

1. **LYL1, GRAP, HHEX, STMN3, FAM113B, MFNG, EIF3G, FXYD5, FSCN1, DNMT1**
2. immune system process (10^-9)
3. regulation of dendritic cell dendrite assembly (10^-9)
4. B cell activation (10^-9)
5. dendritic cell chemotaxis (10^-9)
6. positive regulation of biological process (10^-9)

#### In Tumor Tissue

**Ranked Gene List Top 10**

1. **ASAP2, XIAP, MED13, TBC1D12, FAM120AOS, ACAP2, BCL2L15, MPZL3, FNIP2, SH3RF1**
2. positive regulation of protein localization to endosome (10^-9)
3. negative regulation of cytoplasmic translation elongation (10^-9)
3. forebrain astrocyte development (10^{-5})
4. regulation of protein localization to endosome (10^{-5})
5. 5-methylcytosine catabolic process (10^{-5})

UGT1A10, ZNF774, VPS37B, ASAP2, HK2, LG4, ZG16, EZR, FG4, TSPAN15
1. flavone metabolic process (10^{-4})
2. negative regulation of cytokine secretion (10^{-4})
3. flavonoid glucuronidation (10^{-4})
4. regulation of cellular response to insulin stimulus (10^{-4})
5. negative regulation of cytokine production (10^{-4})

F: Differences between TCF7L1- and TCF7L2-correlated transcriptomes

In Tumor Tissue

| Ranked Gene List | Top 10 |
|------------------|--------|
| STRA6, CDH11, PDGFC, DIO2, ADAMTS12, HEYL, TMEM204, NUAK1, SCG1p, VCAN |
| 1. extracellular matrix organization (10^{-20})
2. extracellular structure organization (10^{-19})
3. animal organ morphogenesis (10^{-11})
4. anatomical structure morphogenesis (10^{-10})
5. developmental process (10^{-9}) |

In Normal Tissue

| Ranked Gene List | Top 10 |
|------------------|--------|
| TSPAN18, FAM127A, FAM127C, CLIP3, EFEMP2, ZBTB47, DACT3, EHD2, CFL2, DBN1 |
| 1. extracellular matrix organization (10^{-9})
2. extracellular structure organization (10^{-7})
3. muscle structure development (10^{-7})
4. developmental process (10^{-7})
5. biological adhesion (10^{-7}) |

In Tumor Tissue

| Ranked Gene List | Top 10 |
|------------------|--------|
| TSPAN18, FAM127A, FAM127C, CLIP3, EFEMP2, ZBTB47, DACT3, EHD2, CFL2, DBN1 |
| 1. cell-cell junction organization (10^{-9})
2. cell junction organization (10^{-9})
3. cardiac muscle cell-cardiac muscle cell adhesion (10^{-9})
4. bundle of His cell-Purkinje myocyte adhesion involved in cell communication (10^{-9})
5. regulation of action potential (10^{-6}) |

Comparison of transcriptome differentially correlated with LEF/TCF gene expression. Pairwise differences between two LEF/TCF genes in transcriptome correlation, comparing TCF7 with LEF1 (A), TCF7 with TCF7L1 (B), TCF7 with TCF7L2 (C), LEF1 with TCF7L1 (D), LEF1 with TCF7L2 (E) and TCF7L1 with TCF7L2 (F). (GO terms listed if p-value < 10^{-3}, shaded if 10^{-4} < 10^{-5}, in normal font if 10^{-6} < 10^{-9}, and in bold if < 10^{-10}). Also see Suppl. Table S1J and Suppl. Table S3.
### Table 4. Comparison between AXIN2- and LEF/TCF-Correlated Transcriptomes.

| Less TCF7 Correlation | Higher TCF7 Correlation |
|------------------------|-------------------------|
| **A: Differences between AXIN2- and TCF7 correlated transcriptomes** | | |
| **In Normal Tissue** | **Associated Gene Ontology Top 5** | **In Tumor Tissue** | **Associated Gene Ontology Top 5** |
| Ranked Gene List Top 10 | AADACL1, FLJ21511, SCYL1BP1, RICS, WDR40A, IQCK, MAK10, DKFZP564C0823, SPTLC1, FAM18B | 1. no GO enrichment found (<=10^-3) | FAM65B, WDFY4, CXC8R5, GAPT, SASJ3, HVCN1, ORSH51, LIMD2, PRKCB, FAM129C |
| Associated Gene Ontology Top 5 | ZNF776, UGT1A4, OR1L6, C10orf99, SIPA1L2, POLG2, CCDC46, EXPH5, ZNF263, PSMA1 | 1. detection of chemical stimulus involved in sensory perception of smell (10^-7) | 1. lymphocyte activation (10^-23) |
| **B: Differences between AXIN2- and LEF1 correlated transcriptomes** | | | |
| **In Normal Tissue** | **Associated Gene Ontology Top 5** | **In Tumor Tissue** | **Associated Gene Ontology Top 5** |
| Ranked Gene List Top 10 | RNF43, FAM84A, PWP2, AIFM3, ATP7B, HIST2H3C, EHF, LPAR2, RICS, ENTPD6 | 1. epithelial cell differentiation (10^-9) | SPP1, C7, APIS2, SLC16A4, COPZ2, DFNA5, KCNMB1, ITGA1, TRPS1, PDGFRL |
| Associated Gene Ontology Top 5 | epithelial cell differentiation (10^-9) | 2. negative regulation of stem cell proliferation (10^-9) | 1. muscle system process (10^-9) |
| | 2. negative regulation of stem cell proliferation (10^-9) | 3. epithelial cell morphogenesis involved in placental branching (10^-9) | 2. muscle contraction (10^-9) |
| | 3. epithelial cell morphogenesis involved in placental branching (10^-9) | 4. developmental process involved in reproduction (10^-9) | 3. relaxation of muscle (10^-8) |
| | 4. developmental process involved in reproduction (10^-9) | 5. negative regulation of epidermis development (10^-9) | 4. relaxation of vascular smooth muscle |
| | 5. negative regulation of epidermis development (10^-9) | | 5. regulation of immune system process (10^-26) |
| **C: Differences between TCF7- and TCF7L2-correlated transcriptomes** | | | |
| **In Normal Tissue** | **Ranked Gene List Top 10** | **Associated Gene** | **In Tumor Tissue** | **Associated Gene** |
| | EHF, C9orf152, GRHL2, LOC57228, MYO6, TOX3, FAM84A, MAP7, ACSM3, IHH | 1. epithelial cell differentiation (10^-9) | FAM127C, PCDH7, ZEB1, GLI3, COP2Z, FAM129A, NEXN, RPBMS2, FERM72, BHM72 | 1. muscle system process (10^-9) |
In Tumor Tissue

| Ranked Gene List Top 10 | In Tumor Tissue | Associated Gene Ontology Top 5 |
|------------------------|----------------|--------------------------------|
| C20orf118, C9orf152, GGH, VWA2, VDAC1, C19orf48, LLGL2, MCM4, PDCD11, RNF43 | 1. nitrogen compound metabolic process (10\(^{-6}\)) | 1. cell adhesion (10\(^{-11}\)) |
|                         | 2. muscle contraction (10\(^{-9}\)) | 2. biological adhesion (10\(^{-11}\)) |
|                         | 3. cell adhesion (10\(^{-11}\)) | 3. organ growth (10\(^{-2}\)) |
|                         | 4. cell adhesion (10\(^{-11}\)) | 4. bone growth (10\(^{-9}\)) |
|                         | 5. anatomical structure morphogenesis (10\(^{-6}\)) | 5. cyclic nucleotide metabolic process (10\(^{-5}\)) |

Comparison of the AXIN2-correlated transcriptome with the TCF7- (A), LEF1- (B), TCF7L1- (C), TCF7L2- (D) -correlated transcriptomes in normal and tumor tissue.

(gene list top 10 genes and top 5 GO terms listed if p-value <10\(^{-3}\), shaded if 10\(^{-4}\)-10\(^{-5}\), in normal font if 10\(^{-6}\)-10\(^{-9}\), and in bold if <10\(^{-10}\)). Also see Suppl. Table S1K.
4. Discussion

4.1. Meta-Analysis of Human Colorectal Cancer Biopsy Transcriptome Studies

After careful selection of individual studies, our meta-analysis reveals a much clearer picture than any individual study, thereby strongly validating our approach. Particularly notable is the dramatic tightening of the confidence intervals of the gene expression of the four LEF/TCF genes and another four selected genes in the meta-analysis compared to those of individual studies (Figure 1). The meta-analysis of correlation of gene expression between eight selected genes (Figure 3) is also much clearer than just comparing individual studies with each other (Figure 2). Generally, our meta-analysis substantiates that correlations are stronger in normal control compared to tumor samples, consistent with the idea of a certain breakdown of controlled gene expression in tumor tissue. An interesting exception to this rule is the stronger association between TCF7 and AXIN2 expression in tumor tissue, which could suggest some specificity in TCF7-mediated WNT/β-catenin signaling in human tumor tissue, supporting earlier such suggestions in the mouse model [23]. Our analysis proved particularly informative when dissecting differences in transcriptome correlation between the four LEF/TCF genes (Table 3) and with AXIN2 (Table 4), comparing tumor with normal tissue samples.

4.2. Differences in LEF/TCF Gene Expression Correlate with Transcriptomes Indicative of Tumor Progression

Our meta-analysis reveals associations that are potentially relevant for tumor progression and possibly metastasis. Cell adhesion-associated gene expression is correlated with TCF7L2 expression specifically in normal tissue, and with LEF1 and TCF7L1 in tumor tissue. Extracellular matrix-associated gene expression is also correlated with TCF7L1 specifically in normal tissue and with LEF1 expression exclusively in tumor tissue. Furthermore, transcripts indicative of angiogenesis are correlated with TCF7L1 in tumor; and transcripts indicative of DNA double-strand break repair and of cell cycle progress with LEF1 in tumor. The expression of NFE2L2, a regulator of p53 and indicator of poor prognosis, is directly transcriptionally regulated by WNT/β-catenin/TCF7L2 in cell culture models of colorectal cancer [46]. In our meta-analysis, NFE2L2 is noticeable for the large discrepancy between positive correlation with LEF1 and negative correlation with TCF7L1 (Supplementary Table S1J), which clearly substantiates the importance of NFE2L2 as an important WNT/β-catenin target gene in human colorectal cancer.

4.3. Indicators of Cell Migration Are Correlated with LEF1, and Likely also Other LEF/TCF Genes

There is clear correlation of transcripts indicative of regulation of cell migration with LEF1 in tumor tissue, consistent with the previously suggested prognostic value of increased LEF1 expression in colorectal cancer for both increased metastasis and for shorter survival prospects of patients [19,20]. Increased LEF1 expression is associated with several types of cancer and has been associated in many tissues with regulation of epithelial-to-mesenchymal transition (EMT) including transcriptional activation of EMT effectors, such as N-Cadherin, Vimentin, and Snail [47]. However, a more complex additional involvement in cell migration of TCF7, TCF7L1 and TCF7L2 remains likely. TCF7L1 expression is correlated, but only in normal tissue, with muscle-associated transcripts, and specifically with expression of the key EMT inducer ZEB1 [48] (Tables 2 and 3). Interestingly, in the mouse, Zeb1 had been described in a mutual feedback regulatory loop with Tcf4/Tcf7l2, rather than Tcf3/Tcf7l1 [49]. The marker for cell migration and known Wnt target HIF1/NEDD9 [50] is correlated in our analysis specifically with TCF7L2 expression (Supplementary Table S1J). EphrinB2 (EPHB2) expression in a mouse model of colorectal cancer is subject to competing positive regulation by Tcf7l2 and negative regulation by Lef1 [51]. Moreover, the related ephrinB3 (EPHB3), a Paneth cell marker and tumor suppressor, has recently been linked in cell line models of human colorectal cancer to transcriptional suppression specifically by TCF7L1 [52]. Our analysis shows EPHB2 and EPHB3 expression, while positively correlated with AXIN2, both negatively correlated with TCF7L1 expression. Among the ephrins, our analysis
highlights the disparity for EPHA1 expression between such negative correlation with TCF7L1 in contrast to positive correlation with TCF7. Increased TCF7 expression in colorectal cancer had previously been shown [22,53] and was recently correlated with cell migration and in extension possibly metastasis [54]. However, the expression of TCF7 in normal and tumor tissue is more complicated than off and on (see below).

4.4. TCF7/LEF1 Gene Expression Correlates with Transcriptome Associated with the Immune System

There is clear correlation throughout our meta-analysis between TCF7/LEF1 expression in normal tissue and transcripts associated with the immune system. However, since TCF7/LEF1 expression is generally low in the control samples from normal tissue (Figure 1), it is likely that the few cells expressing any TCF7 and LEF1 in these isolated normal tissue samples belong to the immune system, and any differences in amount of TCF7/LEF1 transcripts in these samples may reflect varying amounts of immune tissue included in these samples, which would be correlated to immune system-typical transcripts, rather than suggesting a switch in target genes regulated by TCF7/LEF1 in normal and tumor tissue.

4.5. Feedback Regulation of Wnt Signaling Components at the Cell Membrane

Possibly the most surprising insight from this meta-analysis concerns the suggested feedback regulation on Wnt signaling components at the cell membrane. Firstly, there is strong correlation of TCF7L1 with FZD7 expression, particularly in normal but also in tumor tissue (Figures 2 and 3). FZD7 encodes a cell-surface Wnt receptor in intestinal stem cells [41], which has been linked to colorectal cancer [55]. However, there appears to be a pattern; gene expression of FZD8, FZD4, and FZD3 has a similar positive and specific correlation with TCF7L1, while FZD5 expression is negatively correlated with TCF7L1, yet positively correlated with TCF7L2 expression (e.g., Supplementary Table S1J). These correlations are weaker in tumor tissues, particularly with FZD3.

Secondly, AXIN2 expression specifically in tumor tissue is positively correlated with transcripts associated with feedback regulation of the Wnt pathway (Table 2), particularly the Wnt receptor catabolic process (Table 4). Furthermore, there is a correlation with the expression of genes such as RNF43 and ZNRF3, which function as Membrane E3 ligases to promote ubiquitination and degradation of Wnt receptor proteins, including FZD receptor proteins [56], and suggestively, RNF43 and ZNRF3 are frequently found mutated in colorectal cancer [57,58]. LGR5 functions with R-spondin proteins (RSPO) to counteract RNF43 and ZNRF3 and prolong Wnt receptor function at the membrane [56,59]. Our analysis indicates that LGR5 expression is positively correlated with AXIN2, and particularly with TCF7 in normal tissue (Figure 3); also, the related LGR4 is even more strongly correlated with TCF7L2 expression (Table 3, Supplementary Table S2D). NEDD4 and NEDD4L were recently identified as regulators of LGR5 protein degradation [60]. In our analysis, NEDD4 expression (but not NEDD4L) is conspicuous, initially for being differentially correlated with AXIN2 expression, negatively in normal tissue and positively in cancer tissue; and additionally, for being positively correlated with TCF7L1 in normal tissue, which is opposite to AXIN2, but being positively correlated with TCF7 expression in tumor tissue, as AXIN2. These findings are consistent with the suggested role of NEDD4 as a tumor suppressor in colorectal cancer. Among the R-spondin genes, RSPO2 expression is clearly correlated with TCF7L1, but only in normal tissue, while RSPO3 expression is less strongly correlated with TCF7L1, both in normal and tumor tissue.

However, generally, any correlation between transcripts associated with this regulation of Wnt signaling and particularly Wnt receptor catabolic processes is strongest with AXIN2 and TCF7 expression, suggesting specifically for WNT/β-catenin/TCF7 signaling to mediate this feedback regulatory mechanism in tumor tissue. This may relate to mouse organoid culture growth becoming R-spondin-independent with experimental Tcf7 overexpression [23].
4.6. Implications for Molecular Functions of LEF/TCF Proteins and Isoforms

It is clearly difficult to de-convolute the likely molecular functions of LEF/TCF proteins from our transcriptomics meta-analysis; to assess whether there are quantitative differences in the way they function as transcriptional repressors or activators; or whether there are qualitative differences in the direct target genes they regulate, possibly due to additional or altered DNA-binding ability [13,14,61]. However, the strong difference in transcriptome correlation between AXIN2 and TCF7L1 is striking, while TCF7 generally appeared to show the least differences (Table 4). If we accept AXIN2 expression as a proxy for WNT/β-catenin signaling activity, then our analysis is at least consistent with TCF7L1 predominantly functioning as a transcriptional repressor and TCF7 generally predominantly as a transcriptional activator, supporting the concept of a quantitative difference between different LEF/TCF factors [6]. It is also striking that LEF1 and TCF7L1 expression share a correlation with transcripts associated with cell adhesion and ECM organization. These LEF/TCF genes both lack sequences encoding a C-clamp suggesting at least the possibility that the C-clamp-missing LEF1 and TCF7L1 proteins are specifically capable of regulating genes involved in cell adhesion and ECM organization in a qualitatively different way to C-clamp containing LEF/TCF proteins like those encoded by TCF7 and TCF7L2, [14].

Our strict selection procedure resulted in six microarray experiments being considered, since for any meta-analysis, rigorous quality control is of most importance and we think that our unbiased filtering approach provided us with a small but compatible and informative set of studies. We had explored whether the available data could be mined for any information indicating expression of different LEF/TCF isoforms, but that proved impossible. Our analysis therefore only correlates the transcriptome with transcript expression from a LEF/TCF gene, potentially involving several potential gene product isoforms. Furthermore, since our analysis focuses on transcriptional responses, any post-translational functional modification of LEF/TCF proteins [62,63] remains beyond our ability to evaluate.

However, differences in TCF7 isoform expression between normal and tumor tissue have been described [23,63], and our analysis is at least consistent with a different mix of TCF7 isoforms being expressed in normal and tumor tissue. More generally, it is well established that alternative isoforms are expressed from the same LEF/TCF gene [7,64]. Thus, future RNA-seq studies with deep enough sequencing are expected to distinguish different isoforms, as well as reveal regulation by Wnt signaling of alternative isoform expression in potential downstream target genes [65]. It would therefore be very interesting in a future meta-analysis to include RNAseq experiments from an even wider range of databases like cBioPortal [66] to extend the results obtained here. Additionally, future detailed functional investigation is needed and promises to be both important and informative.

5. Conclusions

Our meta-analysis confirms differences in LEF/TCF gene expression in human colorectal cancer tissue and uncovers a correlation of this differential LEF/TCF gene expression with an altered transcriptome, which suggests differences in target gene regulation with likely relevance for tumor progression and metastasis. The analysis also reveals a likely feedback loop in tumor tissue from WNT/β-catenin/TCF7 signaling mediated transcriptional regulation in the nucleus to resulting changes in WNT receptor protein abundance at the membrane. Given the importance of WNT/β-catenin signaling for colorectal cancer and the diversity of known LEF/TCF expression and protein function, our analysis provides an important foundation for future studies to investigate LEF/TCF function and differential isoform expression in more detail.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4425/11/5/537/s1: Figure S1: Principal Component Analysis of selected studies, Figure S2: Principal Component Analysis of de-selected study, Table S1: Transcriptomics Data (Correlation Coefficients) Table S1A: Transcript correlation between eight selected genes (TCF7, LEF1, TCF7L1, TCF7L2, AXIN2, DKK1, FZD7, LGR5); Table S1B: The TCF7-correlated transcriptome; Table S1C: The LEF1-correlated transcriptome; Table S1D: The TCF7L1-correlated transcriptome; Table S1E: The TCF7L2-correlated transcriptome; Table S1F: The
AXIN2-correlated transcriptome; Table S1G: The DKK1-correlated transcriptome; Table S1H: The FZD7-correlated transcriptome; Table S1I: The LGR5-correlated transcriptome; Table S1J: Differences in LEF/TCF-correlated transcriptomes; Table S1K: Differences between AXIN2- and LEF/TCF-correlated transcriptomes, Table S2: Correlated Transcriptome in normal and tumor tissue, Table S3: Comparison of LEF/TCF-correlated transcriptomes, Table S4: Differences between AXIN1- and LEF/TCF-correlated transcriptomes.

Author Contributions: C.-D.M. and S.H. had conceived and supervised this project; C.-D.M. curated the data and carried out some analysis; S.M.L.G., carried out most of the analysis; F.A. wrote an original draft together with Stefan Hoppler. All authors have read and agreed to the published version of the manuscript.

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