Identifying the role of *Transcription Factor 12* (*TCF12*) in colorectal cancer (CRC)

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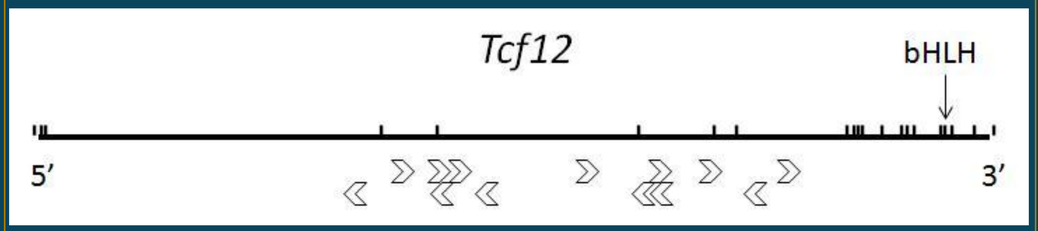
Abstract

In the US, 1 out of 20 people are likely to develop CRC at some point in their life[1]. Less than half of all colorectal cancers are diagnosed before they have spread regionally, at which point treatment becomes difficult[2]. Understanding the genetic mutations that occur throughout CRC progression is vital to improving current treatments or developing new ones. Our lab previously conducted a forward genetic screen in mice that identified the gene *transcription factor 12* (*TCF12*) as playing a primary role in the cascade of mutations that lead to tumor formation[3]. The protein product of *TCF12* is a basic helix-loop-helix protein that binds E-box sequences to enhance gene expression in many different tissues. To determine whether *TCF12* acts as a tumor suppressor gene or a proto-oncogene, our lab is analyzing intestinal tumorigenesis in *Tcf12* conditional knockout mice. Half of these mice also carry a nonsense mutation in the *Apc* gene, which has been shown to cause Multiple Intestinal Neoplasia (Min), a condition characterized by many intestinal tumors. We expect to see more tumors in both cohorts of *Tcf12* null mice if *Tcf12* is a tumor suppressor gene, or fewer tumors in the Apc^{Min} cohort if *Tcf12* is a proto-oncogene. Preliminary results indicate that both cohorts of *Tcf12* null mice show increased tumorigenesis, suggesting that *Tcf12* is in fact a tumor suppressor gene. This differs from recent research showing *Tcf12* overexpression in metastasized CRC tumor cells[4]. We expect that by identifying the exact role of *TCF12* in CRC we will provide a new target for translational research of CRC treatments.

Characteristics of *TCF12*

- Basic helix-loop-helix (bHLH) transcription factor
- Found on chromosome 15 in humans and chromosome 9 in mice
- bHLH domain in exon 18 allows it to bind DNA and dimerize with other bHLH proteins
- Recognizes the hexanucleotide sequence CANNTG referred to as an Enhancer-box or E-box
- Shown to regulate genes involved in lymphocyte development[5] and cell adhesion and migration[4]

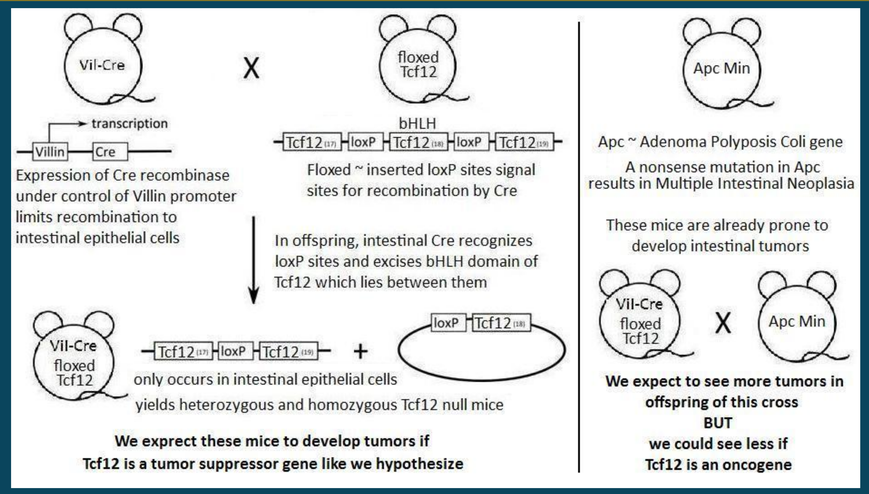
Previous Results Suggested that *Tcf12* is a Tumor Suppressor Gene



Map of *Tcf12* gene and transposon insertions from forward genetic screen[3].

- Transposon consists of promoter and 5′ splice acceptor site
- Chevrons represent transposon insertions and orientation of promoter
- If *Tcf12* was a proto-oncogene, we would have expected to see all chevrons at the 5′ end, pointing right, signifying overexpression of *Tcf12*.
- We hypothesize *Tcf12* to be a tumor suppressor gene because even though the majority of the promoters are oriented positively, their 5′ splice acceptor sites are more likely causing premature splicing of the mRNA transcript, rendering a truncated protein lacking the bHLH domain necessary for function

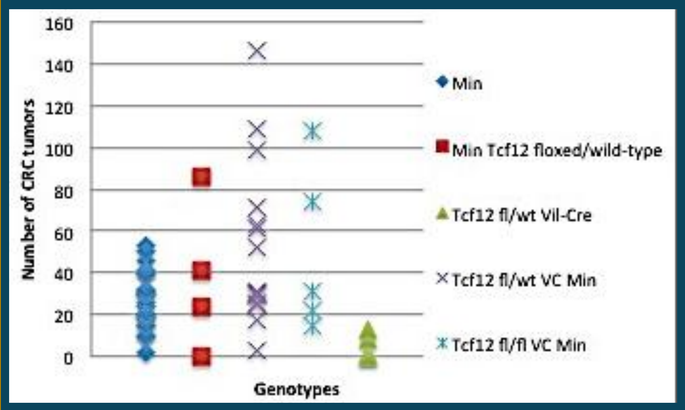
Mouse Breeding



Experimental Procedure

Genotyping by PCR @ 14 days → Necropsy @ 18 months, 120 days (Apc^{Min} mice) or when morbid → Count tumors in intestinal tract using light microscopy

Preliminary Results Suggest *Tcf12* is a Tumor Suppressor Gene



Not shown left: *Tcf12* wild-type mice (zero tumors)

Tcf12 null mice (columns 3, 4, and 5) show more tumors than the corresponding controls (columns 1 and 2, and *Tcf12* wild-type mice, respectively)

Recent Research Has Suggested that *TCF12*'s role is context-dependent

Tumor Suppressor Gene ← → Proto-Oncogene

TCF12 expression ≈ Recurrence-free Survival @ Stage II (Thorsen et al., 2011[6])	*TCF12* overexpression ≈ CRC metastasis (Lee et al., 2012[4])

Moving Forward

Confirmation of genotype and phenotype	• Histology of collected tumors • Tcf12 protein quantification • Loss of heterozygosity of *Apc*
Analysis of human CRC tumors	• Similar to methods stated above and below
Microarray of genes possibly regulated by TCF12	• RAG1 and RAG2 • CDH1 and FN1

Acknowledgments

Starr Lab
- Callie Janik
- Lee Pribyl
- Casey Dorr

Dr. David Largaespada, UMNTC
Dr. Yuan Zhuang, Duke University
Steve Moe
Danette Schaleben (Oct. 19, 1957-May 28,2010)

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