**Summary of proposal:**

Chromosomal aberrations are very frequent in leukemias and several recurring mutations capable of malignant transformation have been described. These mutations usually occur in hematopoietic stem cells (HSC), transforming them into leukemia stem cells. NUP98 gene translocations are an example of such chromosomal aberrations; these translocations produce a fusion protein containing the N-terminal portion of Nup98 and the C-terminal of a fusion partner. Over 75% of Nup98 fusions can interact with chromatin, and lead to changes in gene expression. Therefore, I hypothesize that nup98 fusions act as rogue transcriptional regulators in the cell.

Collecting previously published gene expression data (microarray) from HSCs expressing Nup98 fusions, we can generate some preliminary data to corroborate this hypothesis. Several different fusions affect the expression of similar genes; these are involved in a few biological processes in the cell: embryonic development, immune system formation and chromatin organization. Deregulated genes also present similar transcription factor binding sites in their regulatory regions. These putative regulatory transcription factors are highly interconnected through protein-protein interactions and transcriptional regulation among themselves, and they have important roles in cell cycle regulation, embryonic development, hematopoiesis, apoptosis and chromatin modification.

In this proposal I will extend this knowledge to all Nup98 fusions and identify the mechanisms behind the effects they have in gene expression. Using HSCs expressing a GFP tagged version of all known NUP98 gene fusions as a model system, I propose two Aims in order to achieve a network level understanding of the role NUP98 translocations play in leukemogenesis:

**- Aim 1: Constructing the interactome of Nup98 fusion oncoproteins.** Very few protein-protein interactions have been described for nup98 fusion proteins, most of which involve chromatin modifiers that can affect gene expression; in addition, several fusion proteins can interact with chromatin, but only one locus where this interaction occurs has been described. This indicates it is imperative that we expand our knowledge on the subject. This is address in two ways in Aim 1: 1) determining protein interaction networks for leukemogenic nup98 fusions – using affinity purification of protein complexes followed by high resolution liquid chromatography and tandem mass spectrometry for protein identification; 2) mapping genome-wide nup98 fusions interaction with chromatin – using a chromatin immunoprecipitation protocol followed by deep sequencing of all DNA regions with enriched binding to the fusions. The results of these two experiments will generate interaction networks of nup98 fusions in the cell. Careful analysis of these networks will identify altered interactions and several novel connections of these fusions to cellular pathways, hinting at their functional role. Given our initial hypothesis that nup98 fusion may act as rogue transcriptional regulator, Aim 2 attempts to further our understanding of the functional role these fusions have within this cellular network.

**- Aim 2: Effects of NUP98 translocations in the transcriptome of HSCs.** The effect of some nup98 fusions in gene expression has been explored through microarray experiments. However, several still remain to be evaluated, and broader comparisons between different fusions is still lacking. This will be achieved by purifying the complete RNA pool present in HSCs expressing different nup98 fusions and submitting all samples to deep sequencing. Results will provide information on the expression level of several mRNAs, splice isoform abundance, RNA editing events, preferential allele expression, non-coding RNA abundances, and presence of mutant transcripts. Merging these data with those obtained in Aim 1 will generate a regulatory network of gene expression, and help clarify the mechanisms by which nup98 fusions affect gene expression as well as the consequences this deregulation has in the cell.

Achieving the Aims described here I will broaden the understanding of how nup98 fusions fit in the cellular landscape and how they lead to the disease phenotype. This integrated differential disease network will be the scaffold from which to tease out affected pathways and identify important therapeutic targets. It will generate several novel testable hypotheses on functional principles and mechanisms underlying leukemia development, and it can be expanded as these new questions are answered making it a more robust and detailed model of this disease.