Tumor-Mediated Fibrinolysis as a Mechanism and Therapeutic Target in Osteosarcoma Metastasis

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SPECIFIC AIMS

Background: Lung metastases remain the principal driver of mortality for patients with osteosarcoma. Five-year survival is below 30% for these patients – a statistic that has not improved in over 50 years. There is a well-documented relationship between cancer and coagulation, yet the role of coagulation in driving osteosarcoma metastases remains underexplored. We have recently shown that pharmacologic depletion of fibrinogen accelerates osteosarcoma tumor growth and angiogenesis *in vivo*, suggesting that fibrin(ogen) acts as a tumor-suppressive barrier. Whether the fibrin matrix governs *metastatic* dissemination remains unknown.

Preliminary Data: Using the MOSJ model (an orthotopic, syngeneic model of osteosarcoma in immunocompetent C57BL/6 mice) we found that wildtype (WT) animals with normal fibrinogen ($Fga^{+/+}$) do not develop pulmonary metastases. This is consistent with previous studies, as **spontaneous lung metastases have never been reported in any MOSJ model**. We initially hypothesized that implanting MOSJ cells into $Fga^{EK/EK}$ mice (which have circulating fibrinogen that is genetically modified so it cannot be polymerized into fibrin matrices) would mimic our previous pharmacologic depletion studies. However, these mice had primary tumor growth and overall survival similar to WT mice. Unexpectedly, $Fga^{+/EK}$ heterozygous mice, producing a 50:50 mixture of normal and non-polymerizable fibrinogen, developed massive primary tumors and ***lethal spontaneous lung metastases****,* leading to significantly shorter overall survival. These findings suggest a dominant-negative mechanism in which nonpolymerizable fibrinogen monomers poison the formation of functional fibrin clots, rendering them more vulnerable to tumor-mediated fibrinolysis, invasion, and metastases.

Current Hypothesis: Osteosarcoma metastases are not contained simply by the presence or absence of a fibrin barrier; rather, they are driven by a balance of the fibrin barrier vs the associated enzymatic activity triggered by fibrinolysis in the tumor microenvironment (TME). In $Fga^{+/EK}$ heterozygous mice, incorporation of non-polymerizable fibrinogen monomers into clots creates a fibrin matrix that is present but more vulnerable to tumor-induced fibrinolysis. The resulting cycle of fibrin deposition and fibrinolytic degradation generates pro-angiogenic and pro-metastatic signals, such as liberation of matrix-sequestered growth factors, that promote angiogenesis and metastatic dissemination. This "worst of both worlds" phenotype explains why $Fga^{+/EK}$ heterozygotes develop more aggressive disease than either wild-type mice (whose intact fibrin matrix resists degradation) or $Fga^{EK/EK}$ homozygotes (who lack a fibrin substrate to induce fibrinolysis). We hypothesize that this fibrin-fibrinolysis axis is recapitulated in human osteosarcoma and represents a targetable mechanism for preventing metastasis.

Aim 1: Determine if the metastatic phenotype of $Fga^{+/EK}$ heterozygous mice is driven by dominant-negative poisoning of the fibrin clot or fibrin haploinsufficiency. We will perform orthotopic injections of MOSJ cells across an allelic series of genetically engineered mice: WT ($Fga^{+/+}$), $Fga^{+/-}$, $Fga^{-/-}$, $Fga^{EK/EK}$, $Fga^{+/EK}$, and $Fga^{-/EK}$. Primary endpoints: survival, lung metastasis burden, primary tumor growth. The **decisive comparison** is $Fga^{+/-}$ vs. $Fga^{+/EK}$: both genotypes carry one functional fibrinogen allele, but only $Fga^{+/EK}$ mice incorporate non-polymerizable monomers into forming clots. If $Fga^{+/-}$ mice are protected while $Fga^{+/EK}$ mice develop lethal metastases, the phenotype is attributable to dominant-negative poisoning, not haploinsufficiency. Secondary comparisons, such as $Fga^{EK/EK}$ vs. $Fga^{-/-}$, will establish if fibrinogen itself has a protective function.

Aim 2: Determine whether tumor-mediated fibrinolysis is required to breach the compromised fibrin barrier and drive metastasis. *Genetic (primary):* We will cross plasminogen knockout mice ($Plg^{-/-}$), which lack the primary enzyme required for fibrinolysis, with $Fga^{+/EK}$ mice to generate $Fga^{+/EK}$; $Plg^{-/-}$ compound mutants. MOSJ injection will compare $Fga^{+/EK}$; $Plg^{+/+}$ (lethal phenotype) to $Fga^{+/EK}$; $Plg^{-/-}$ (predicted rescue). If plasminogen loss rescues $Fga^{+/EK}$ metastasis, it demonstrates that the compromised fibrin barrier can only be breached when tumor cells have access to fibrinolytic machinery, meaning poisoned fibrin is necessary but not sufficient; active fibrinolysis is required. *Pharmacological (translational):* $Fga^{+/EK}$ mice bearing MOSJ tumors will be treated with tranexamic acid (TXA), an FDA-approved anti-fibrinolytic drug already used in orthopaedic surgery. If TXA phenocopies the $Plg^{-/-}$ rescue, it suggests a clinical strategy for suppression of osteosarcoma metastasis.

Aim 3: Determine if the fibrin-fibrinolysis axis is recapitulated in human patients with osteosarcoma. Our murine data generate testable predictions about the human osteosarcoma TME. Leveraging an established REDCap clinical database and tissue repository comprising over 50 patients with matched primary and lung metastatic osteosarcoma treated at our institution, we will perform multiplexed spatial proteomics (CODEX/PhenoCycler) with a 50-marker panel including a hemostatic module focused on coagulation and fibrinolysis, alongside immune, vascular, and stromal markers. We predict that primary tumors will show peri-tumoral fibrin deposition inversely correlated with invasion; plasminogen activator and receptor expression will be enriched at regions of fibrin degradation; and lung metastases will show reduced fibrin matrix integrity with elevated fibrinolytic markers. **IMPACT:** By integrating a fibrinogen allelic series, genetic and pharmacological fibrinolysis inhibition, and multiplexed spatial proteomics on human samples, this proposal will establish the hemostatic tumor microenvironment as a therapeutic target in osteosarcoma metastasis.